Art and Science of Breastfeeding & Beyond

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Foreword
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CHAPTER 3

Anatomy and Physiology of Breast

Padmashri S Kudachi

"Breastfeeding is the priceless gift of the mother to the baby"

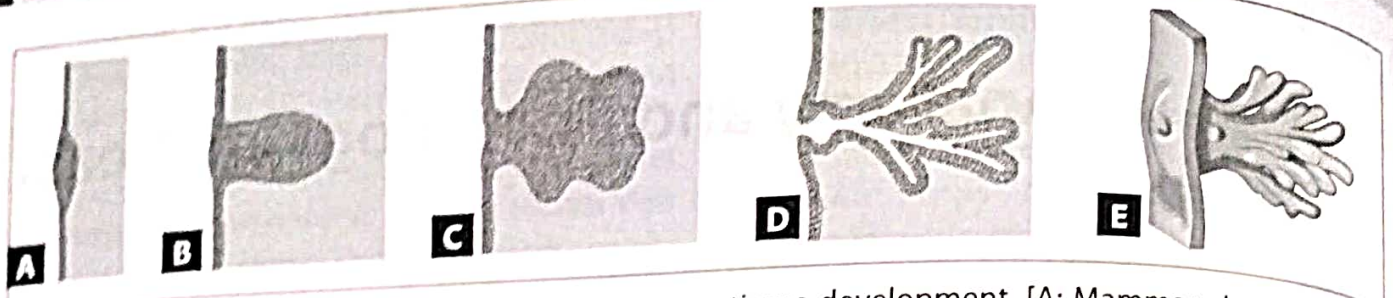
INTRODUCTION

The mammary gland is a unique feature of humans and all mammals which produce milk for the nourishment of offspring. It consists of glandular and fat tissue supported by connective tissue. The breast undergoes significant morphological and functional changes during various phases of a woman's life. This chapter describes the dramatic developmental changes of the breast observed during the fetal stage, puberty, pregnancy, lactation, and involution. Further the role of hormones, lactation, and recent updates related to this topic are discussed.

MAMMARY GLAND DEVELOPMENT

Embryology (Fetal stage)

The two compartments of breast include epithelial component originating from ectoderm and surrounding stromal part from mesoderm.[1] The ectoderm forms the ducts and alveoli, whereas the mesenchyme forms the connective tissue and its vessels. Early development of human mammary gland gets initiated at 4th week of intrauterine life as a localized thickening of the ectoderm.[2] The mammary band is identified at 5–6th week of intrauterine life as milk line (thickened ectoderm), also known as galactic band seen extending from axilla to groin. The mammary band involutes during 6–7th week, except for the thoracic region which presents as four-to-six cell wide thickening representing the future breast location. Mesenchymal cells around epithelial bud differentiate into mammary mesenchyme. During 7–8th week, the mammary bud invades into the mesenchyme beneath and starts branching as it reaches the fat pad. Between 10–12th weeks of gestation, mammary epithelial buds form and start showing different differentiation stages[3] **(Figs. 1A to E)**. By 13–20th week, 15–25 epithelial cords which later with multiple openings (galactophores) join at each nipple; among them, 8–15 ducts are patent. These cords branch and canalize, leading to formation of primary milk (lactiferous) ducts by 32 weeks of gestation.[2,3] The term branching morphogenesis refers to the tree-like ductal branches extending from epithelial bud.[4] In 80–90% of newborn infants, the ducts may contain milk-like secretion called the "witch's milk"; due to influence of maternal hormones circulating in fetal circulation, fetal pituitary prolactin (PRL) gradually reaches its peak levels at term pregnancy.[2,5] Around a



Figs. 1A to E: Various embryonic stages of mammary tissue development. [A: Mammary bud (5th week of gestation); B: Downward growth of mammary bud; C: Secondary bud formation (5–12th week); D: Mammary lobules development (12 week); E: Further growth of lobules, branching of duct) converging at inverted nipple (>12 weeks)]

Source: Adapted from Jesinger RA. Breast anatomy for the interventionalist. Tech Vasc Interv Radiol. 2014;17(1):3-9.

month after birth, the newborn's mammary gland regresses with low PRL levels.[3]

At birth, the ducts open on the outer surface of the skin as a small pit. The nipple everts during proliferation of underlying mesenchyme and the skin surrounding the nipple forms the areola after proliferation. Duct branching and terminal lobule development is seen till the age 2, later the lobules involute and breast remains dormant until pubertal hormonal influence but grows in proportion to the other tissues till puberty.[6,7] The embryonic development of mammary gland is hormone independent, while later stages are influenced by multiple hormones.[8] At birth the gland consists of number of blind-ended tubes and terminal duct lobular units (TDLU).[9-11]

The areola around the breast nodule is formed by the ectoderm during the 5th month of intrauterine life. The infants born before term fail to develop the breast nodules, which indicates the importance of the intrauterine environment for breast development.[11]

Terminal duct lobular unit is a group of acini originating from one terminal duct **(Fig. 2)**, which is the functional unit of the breast. On the basis of differentiation, four types of TDLU are recognized. Type I lobules are most primitive, seen among adult nulliparous breast (18–20 years of age), and have great number of receptors of estrogen and progesterone with complete ductal and stromal maturation. Type II and III show terminal duct with many branches, forming more number of acini, characterized by less proliferative index. Type IV lobules are present only in lactating women. Postmenopause, breast shows Type I TDLU.[8]

Pubertal Stage

Thelarche, the phase where adult breast development begins is the indicator of the onset of puberty, observed between 8–13 years of age, about 2 years before menarche[12,13] **(Table 1)**. During initial anovulatory cycles after the menarche, the breast tissue is exposed to uninhibited action of estrogen known as estrogen window[14] which causes ductal epithelial thickening elongation of existing ducts, formation of secondary ducts by branching, and clusters of alveolar budding, forming lobules.

With the onset of ovarian cycle at puberty, in follicular phase of menstrual cycle, the ducts in the mammary gland have little or no lumen, while in midcycle, the lumen epithelium becomes taller due to the effect of estrogen. The vascularity of the breast is known to increase during the ovulation

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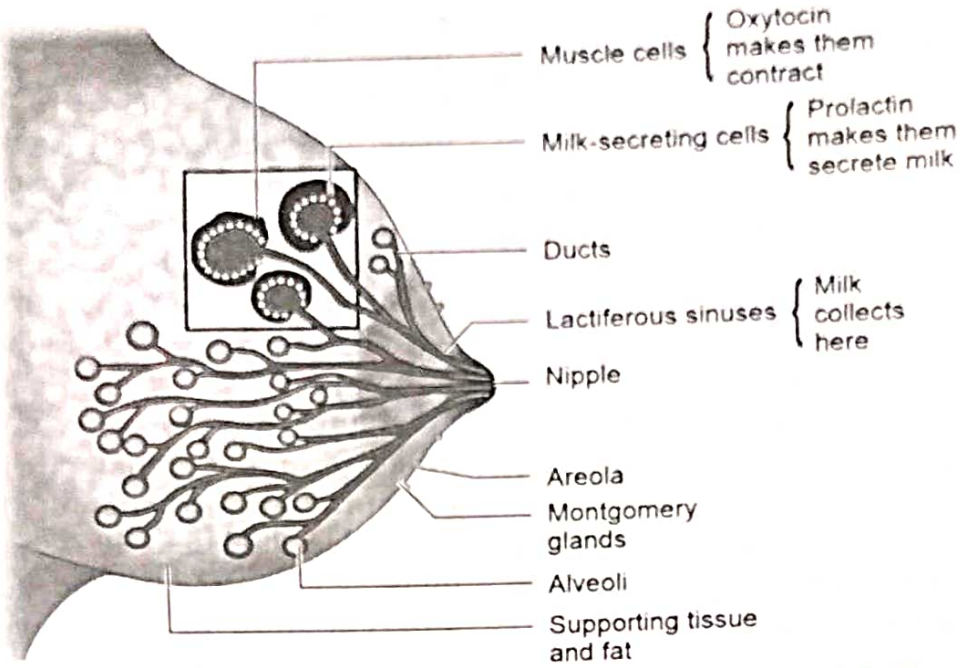

Fig. 2: Schematic diagram indicating terminal duct lobular unit (TDLU).

TABLE 1: The developmental stages of breast described by Tanner.[18]

Stages	*Age (years)*	*Features in the breast*
1	10 or <10	Slight elevation of only papilla, no palpable glandular tissue (preadolescent)
2	10–11.5	Breast and papilla both elevated, palpable breast bud (first pubertal sign)
3	11.5–13	Enlargement of the breast beyond areolar region
4	13–15	Further enlargement of areola and papilla form a secondary mound above the breast
5	15 and above	Adult breast, areolar mound recession to the general contour of the breast, nipple protrusion

phase of menstrual cycle.[15] The luteal phase shows second phase of mammary gland growth under the influence of progesterone secreted by corpus luteum which is termed lobuloalveolar growth, which attains full growth during pregnancy.

In luteal phase, duct cells differentiate to be able to secrete; blood flow in the stromal tissue increases, leading to discomfort caused by edema and hyperemia of the breast experienced during premenstrual period. The lobules with alveoli reach maximum size and number in secretory phase, during 21–27 days of menstrual cycle, correlated with serum progesterone levels.[5,12] The breast lobules which show greater proliferation rate exhibit more receptors for both estrogen and progesterone.[5] The lobuloalveolar structure undergoes regression with the hormonal withdrawal with the onset of menstrual bleeding and regains growth during luteal phase dominated by progesterone.[16] These cyclical changes continue and contribute to the new development and new budding in lobule with each menstrual cycle till the age of 35 years.[5,17]

Role of Hormones

Secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus during puberty stimulates follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Key regulator of pubertal breast before menses is estrogen, secreted by ovary, which acts on estrogen receptor-alpha (ERα). Estrogen action depends on the growth hormone and in turn on insulin-like growth factor-1 (IGF-I) in the mammary gland.[19] Estrogen also favors the action of FSH and LH for normal breast growth.[2,20]

Hence, the hormones, estrogen, growth hormone, IGF-I, and epidermal growth factor are known to cause ductal elongation. Progesterone, PRL, and thyroid hormone favor duct branching and more alveolar formation.[2] PRL increases progesterone secretion from the corpus luteum and acts indirectly by facilitating progesterone's actions on the breast. Insulin by acting on IGF-I receptors may also contribute to ductal or lobuloalveolar development, but it is not essential.[12,16] Triiodothyronine, glucocorticoids, and vitamin D3 are the other hormones involved in breast development during puberty.[5,11]

Breast Changes in Pregnancy

Mammary gland undergoes dramatic changes and full development in pregnancy. First trimester of pregnancy shows increased distal duct proliferation to form TDLU-4 with more lobules and alveoli. In second trimester of pregnancy, the mammary gland prepares itself for milk synthesis called lactogenesis I, characterized by increased cell organelles and cytoplasmic lipid droplets. During pregnancy, high PRL levels will not cause milk production due to the suppression by progesterone.[21] In the last trimester, fat droplets will get collected in the epithelial cells and the acini gets filled with colostrum. More of glandular tissue replaces the connective tissue and fat in the breast. Vascularity of the mammary tissue increases markedly, attributing to large volume of breast.[12,13]

The breast size in pregnancy does not determine milk production of lactation, neither the preconception of breast size is related to the magnitude of increase in size during pregnancy.[8,22] The changes during pregnancy are influenced by estrogen, progesterone, adrenal corticoids along with protein hormones of the PRL, and glycoside hydrolases (GH) family.[23,24] During midpregnancy, insulin increases secretory differentiation of mammary gland and decreases its proliferation.[16,25]

Breast Changes During Lactation Phase

Metabolic demand increases significantly in lactation with need of 25% of daily energy intake for milk synthesis.[3] The important changes seen from pregnancy to lactation are increase in alveoli with secretory capacity and peak levels of gene expression for the synthesis of lipid, enzymes, lactose, and milk protein seen during 3–4 days postpartum.[23,24]

Metabolic hormones including insulin and thyroxin prepare the myoepithelial cells to synthesize mature milk composed of fat, lactose, and protein in 30–40 hours postpartum phase.[26] The onset of milk secretion around parturition is because of fall in levels of progesterone and further maintained by PRL and glucocorticoid.

Key hormone in regulating lactogenesis is PRL which has receptors on the alveolar basal membrane and secreted in response to infant suckling or stimulation. However, serum PRL levels do not control milk production but infant suckling and nipple stimulation do, which explains the basis of skin-to-skin (S2S) theory. The feedback inhibitor of lactation (FIL)

is a whey protein identified in breast milk that acts as the local negative feedback mechanism for milk synthesis.[22] Insulin, thyroid hormone, estrogen, and GH are known to enhance the action of PRL. Other hormones involved in regulation of lactation are estrogen and oxytocin.

The hormones also increase the mammary blood flow. There are also increased stromal lymph vessels to help clearance of particles and fluid from breast.[27]

The phases of lactation[28] are as follows:

1. *Mammogenesis:* Development and proliferation of the mammary tissue under the influence of estrogen and progesterone to stimulate mammogenesis and simultaneously inhibit PRL.
2. *Lactogenesis:* The proliferative state of breast in pregnancy gains secretory function during lactation is called lactogenesis.[29]
3. *Galactopoiesis:* Further maintenance of milk secretion[28]
4. Involution.[30]

Lactogenesis I is observed at 15–20 weeks of gestation with the ability to produce milk. Colostrum secretion begins and collects in the alveolar lumen at this phase. This colostrum contains no fat unlike milk secreted in postpartum period.[31,32] After child birth, removal of placenta causes rapid fall of progesterone, resulting in lactogenesis II (L-II).

Large amount of milk secreted during first 3–4 days after parturition known as "milk coming in" in the first 40 hours,[16] which is accompanied with great rise in blood flow. The oxygen and glucose uptake also increases in breast tissue. Milk volume increases from 100 mL/day—an initial value to approximately 600 mL/day by 4–5 days.[21,33] The maximum amount of storage of milk in breast ranges from 80–600 mL.[22] The normal, healthy, lactating woman can produce up to 1 liter of milk in a day.[12]

The relation of blood flow and outcome of milk is observed and the ratio[3] is described as 500:1.

Lactogenesis I and II occur irrespective of initiation of breastfeeding, independent of any stimulus, such as suckling or pumping of milk.[34] Further, the lactation is sustained by the epithelial cells following the L-II phase known as galactopoiesis (lactogenesis-III). Both lactogenesis I and II are controlled by hormones, while the galactopoiesis is regulated, mainly maintained by regular milk removal (autocrine control).[22,35]

Important pathways by which milk components are secreted in the lumen are exocytosis for proteins, fat by forming milk fat globule, transcytosis for immunoglobulins, and secretion for water and ions.[16]

Involution

Infrequent breastfeeding after the start of weaning, apoptosis of epithelial cells sets in and the gland regains nonlactating form; however, the breast structure is not similar to the nulliparous state.

Low levels of PRL, GH, and IGF-I are observed in this stage.[30,36] The secretory parts in the breast undergo regression, lobules decrease in size with decreased alveoli without involving ducts, unlike the involution of menopause, where both alveoli and ducts reduce in number **(Fig. 3)**. The first phase of involution is reversible, as suckling will restart the milk flow, while in the second phase, women experience loss of lactation secondary to absent suckling for more than 48 hours.[12,37]

Milk Ejection

Release of stored milk is called milk let down or milk ejection reflex.[12]

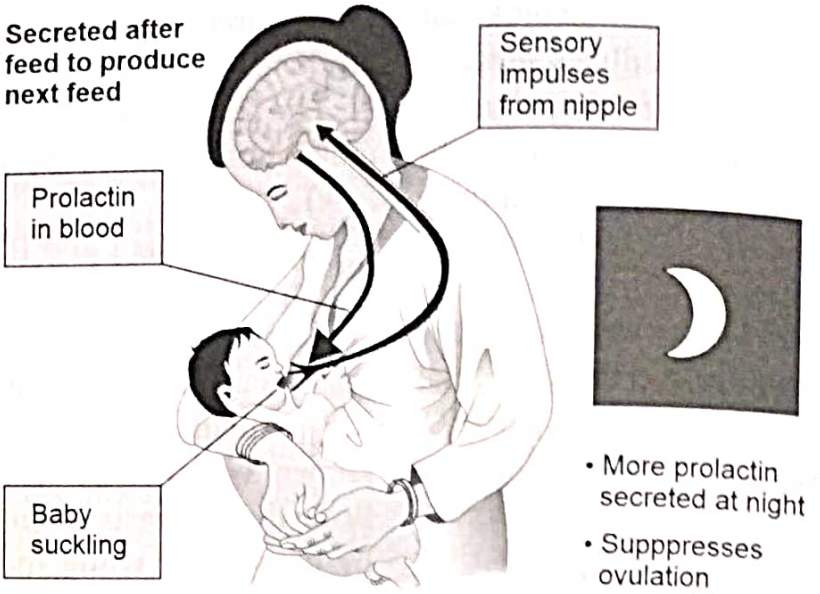

Fig. 3: Prolactin reflex.

Oxytocin is the main hormone regulating this reflex, which is released from the maternal neurohypophyseal-posterior pituitary axis by neuroendocrine mechanism. Physical stimulation of the nipple areolar complex causes activation of somatosensory afferent neurons (T4, T5, and T6 nerve roots).[38] Oxytocin acts on receptors of myoepithelial cells causing expulsion of milk in the ducts, leading to increased intraductal pressure[39] dilation of duct and increased milk flow rate **(Fig. 4)**.[40] Such multiple milk ejections may occur during feeding the infant. Milk secretion is influenced by emotions; stimulated with hearing a cry of baby, preparing to breastfeed and inhibited by factors such as anxiety, embarrassment, and pain.[22,41] Neuropeptide Y secreted from sympathetic fibers as a result of stress may inhibit this reflex, causing less milk outcome.[42]

Menopause

Between the age of 45–55 years, breast shows moderate reduction of glandular tissue followed by the regression of breast stroma which is replaced by fat and apoptosis of glandular epithelium. Both ducts and lobules decrease in the mammary gland, showing more of lobule type I and reduced number of lobule type 2 and 3 in menopause.

Menopause is associated with low levels of estrogen and progesterone and rise in ovarian androgens, testosterone androstenedione, and dehydroepiandrosterone.[12]

BREAST—GROSS ANATOMY

This section includes the structure of breast, originally described by Cooper in 1840,[43] along with the newer findings observed by few studies in later years.

Breast is hemispherical or teardrop-shaped structure composed of glands (secretory) and adipose tissue held by connective tissue, located on the chest between the 2nd and 6th ribs, lateral to sternum and medial to the anterior axillary line. It lies anterior to the deep pectoral fascia with the superior pectoral fascia covering it ventrally. The muscles pectoralis major, serratus anterior, and inferiorly, external oblique situated underneath the deep pectoral fascia. The suspensory ligaments of Cooper provide structural support to the breast parenchyma by connecting both superficial and deep pectoral fascia.[44]

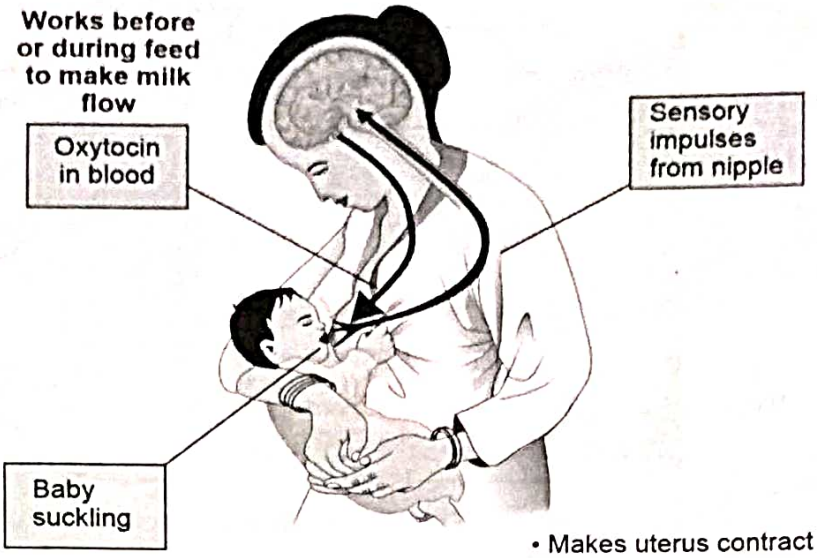

Fig. 4: Oxytocin reflex.

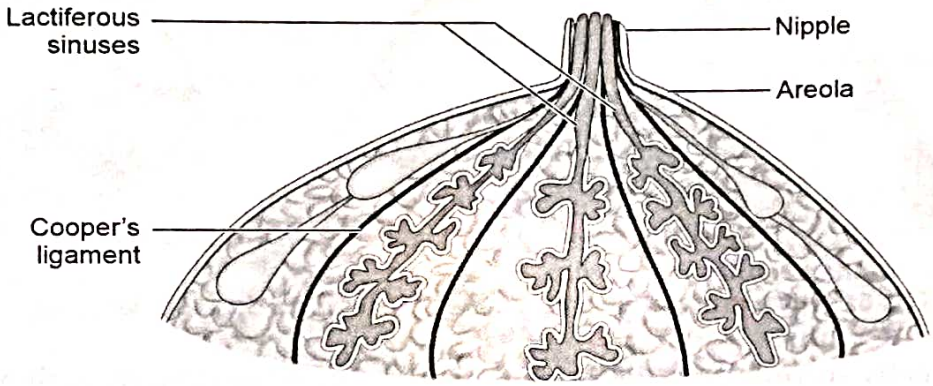

Fig. 5: Cooper's ligament and division of lobes.

Retromammary space, which separates the breast with the underlying deep pectoral fascia, allows the mobility of the breast tissue.[45] Cooper's suspensory ligaments divide the mammary gland into 15–20 sections, also called as lobes[46,47] composed of lobules containing 10–100 alveoli of approximately 0.12 mm in diameter[3,48] **(Figs. 5 and 6)**. The alveoli are lined by secretory epithelial cells and connected to very small ducts that join to form larger ducts which drain the lobules. These larger ducts finally join together to a single duct coming out from each lobe. Such 15–20 ducts converge toward the nipple, terminating at its orifice on the surface of the nipple, out of which only 7–12 ducts appear to be generally patent. Adipose tissue in the breast is present more between lobes than within lobules.[48] Recent ultrasound study has shown that the dilation of the duct located near alveolar area known as "lactiferous sinuses" are not seen, which were believed to store milk before milk ejection. Instead, it is found that the ducts only distend during milk ejection, indicating that the ducts transport milk rather than store it.[49]

Two layers of epithelial cells form the lining for each duct—cuboidal cells capable of differentiating into milk secretory cells during lactation, while the outer contractile

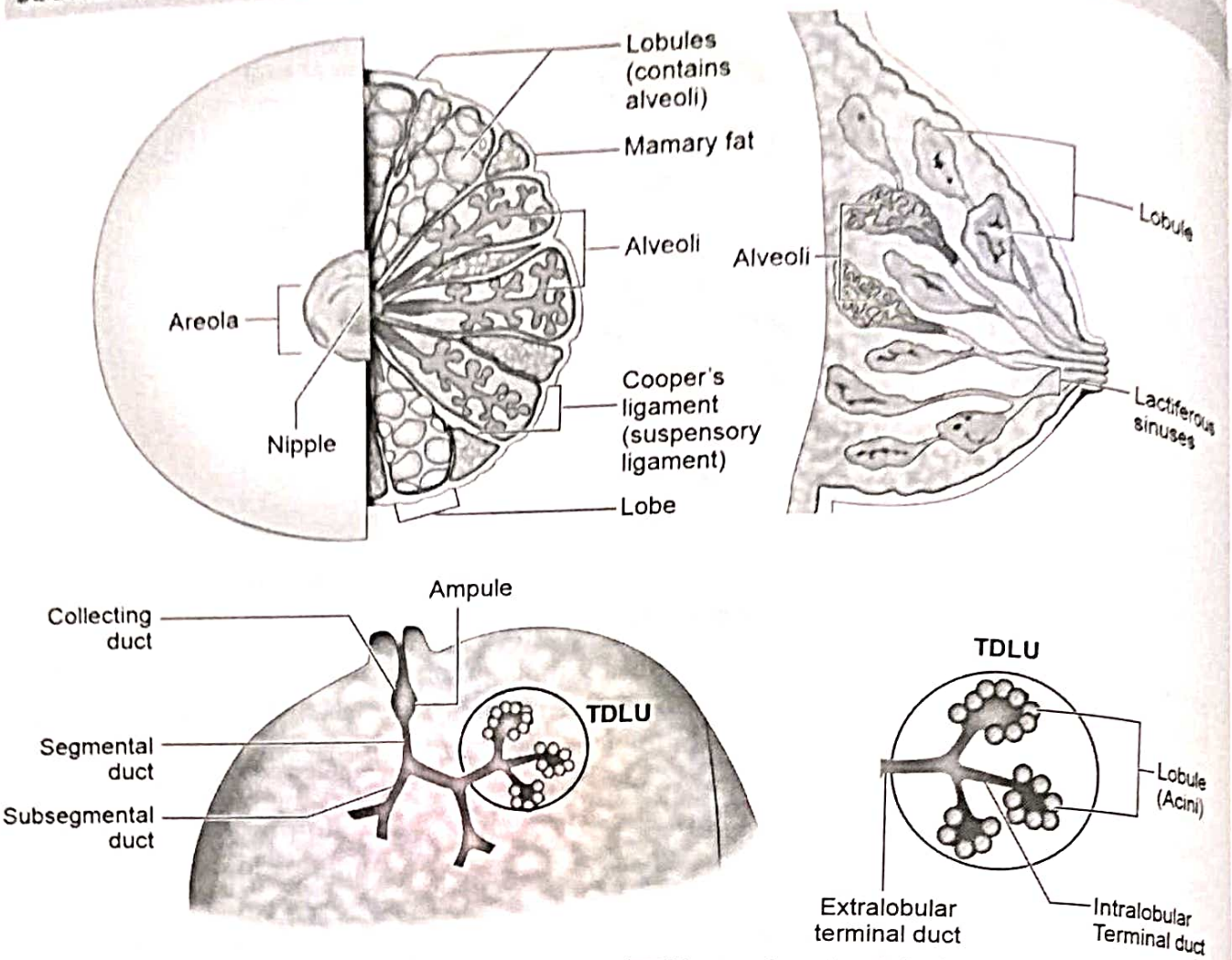

Fig. 6: Structure of mammary tissue. (TDLU: terminal duct lobular units)

myoepithelial cells surrounding the lumen have properties of smooth muscle cells.[3] Basement membrane is adjacent of myoepithelial layer on the outer side[45] **(Fig. 7)**. Luminal cell function is to generate milk-secreting cells during lactation and myoepithelial cells help in expulsion of milk.[50]

The periphery of the areola has Morgagni tubercles, the elevations formed by the duct openings of the modified accessory glands known as Montgomery glands which are capable of producing milk.[51]

Blood Supply

Major vascular supply (60%) of the mammary tissue is by internal thoracic artery (internal mammary artery), lateral thoracic artery supplies 30% and minor 10% of the blood supply is contributed by the thoracoacromial, intercostals, subscapular, and thoracodorsal arteries.[22,44] The venous drainage is to the intercostal, axillary, and internal thoracic veins.[44]

Innervation

The nerve supply to the breast is by anterior and lateral cutaneous branches of intercostal nerves IV, V, and VI. The nipple is mainly innervated by fourth intercostal nerve.[46,52]

The areola and the nipple area are very sensitive because of the cutaneous nerve plexus which is located underneath the areola.[45,51] Sympathetic fibers innervate the circular muscles of the nipple and the smooth muscle around the ducts and blood vessels. There is no parasympathetic supply to the breast as

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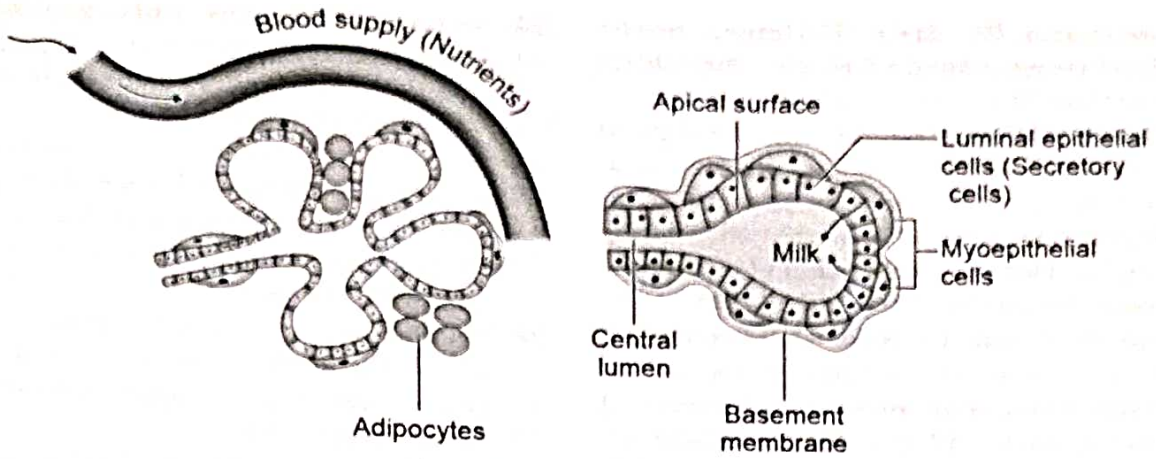

Fig. 7: The alveolar structure.

the mammary gland secretion is regulated by hormones.

Lymphatic Drainage

Approximately, 75% of the lymph from the mammary gland (medial and lateral portions) is received by axillary lymph nodes. The internal mammary nodes drain largely from the deeper region of the breast.[53] Lymphatic vessels may cross the central plane and connect with the contralateral breast.[3] Densely arranged lymphatic vessels are located underneath the areola in the subcutaneous layer, also known as Sappey's subareolar plexus.[51,54]

RECENT UPDATES

Recent studies have not found the presence of dilated duct (lactiferous sinuses), the function of which is to transfer and not storage of milk as believed in the past.[55] Milk flows out by the vacuum created in the infant's mouth rather than duct peristalsis. These ducts are few in number, branch near the nipple, are superficially located, and easily compressible.[56,57]

KEY MESSAGES

- Positive state of mind is critical for the efficient lactation.
- Prolactin and oxytocin are the main hormones involved in synthesis and ejection of breast milk respectively.

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CHAPTER 8

Maternal Nutrition and Quality of Breast Milk

Padmashri S Kudachi

"The mother has to have enough food in order to produce enough milk in order to breastfeed, but she has to know that she should breastfeed. That's an education issue".

—Catherine Bertini

INTRODUCTION

In the process of evolution of human beings from Homo sapiens, the quality of food we consume today has changed tremendously under the influence of western diet and more processed food. It is very critical for the mothers to choose and eat wisely as it affects the growth and development of the fetus in the womb and breastfed babies in the postpartum phase as well. Very few studies have observed the direct link between the maternal diet and composition of the breast milk with varied conclusions. The present chapter will discuss the association of maternal diet and breast milk composition in terms of macronutrients and micronutrients.

Mother's milk is the best nutrition for the newborn; hence, nutrition of the mother is of prime importance. The composition of human milk is influenced by numerous factors apart from diet, including mother's lifestyle, gender of the infant, genetics, environment, etc.[1]

The breast milk consists of 60–75 kcal /100 mL of energy, carbohydrate (lactose 6.9–7.2%), protein (0.8–0.9%), and fat (3–5%). It also contains minerals and nitrogen compounds.[2]

MACRONUTRIENTS

Dietary Fat and Quality of Breast Milk

Maternal diet rich in fat, carbohydrate, and protein increases milk fat concentration.[3] It is observed that consumption of macronutrients in the maternal diet does not affect the breast milk macronutrient content; however, fatty acid levels are linked to immediate food intake.[4,5] Diet rich in edible oils and fish oils during pregnancy is known to increase these essential fatty acids.[6] High consumption freshwater fish in women showed more than recommended levels of omega-3 fatty acids in the breast milk, which makes us reanalyze the present recommendations of the infant formulae food and optimize the maternal intake.[7,8]

Both the monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) in breast milk are correlated with intake in the mother.[9-11] Supplementation of docosahexaenoic acid (DHA) in mother

increases the breast milk fatty acid levels proportionately[12] and the PUFAs significantly increase in mature milk.[13-17] Administration of arachidonic acid (AA) in combination with eicosapentaenoic acid (EPA) and DHA is known to increase these fatty acids in the breast milk compared to administration of AA alone.[18]

It is shown that obese mothers secrete more saturated fatty acids and less of PUFA in the breast milk compared to normal weighing mothers, which affects weight gain of newborns in the initial few months, which may also influence the health of the child later.[19] The fetal nervous system is very sensitive to the maternal dietary supply of essential fatty acids and long-chain fatty acids,[20,21] such as omega-3 and omega-6 fatty acids, known to benefit the growth of the newborn and its brain development.[22] Low-level fatty acids in the maternal diet will adversely affect the fetal brain; hence, quality of mother's diet is important.[23] This finding is supported by many studies which demonstrated better psychomotor skills at the age of 30 months and intelligence of children in long term, when mothers were given DHA supplement.[24,25] Breast milk fatty acid is also dependent on the protein intake of mother as both metabolism of lipid and protein are linked. Low intake of protein is known to cause decreased supply of DHA from mother to fetus, causing adverse effects on the brain development.[26]

Energy Content

Maternal nutrition also influences the energy levels along with fat composition in breast milk.[27] High dairy food consumption increases the breast milk lipid content[28] and high lipid leads to more energy output.[29,30] Few studies have shown that low energy consumption in the mother affects the milk production, while majority research has suggested no change in milk production despite low provision of calories in the mother's diet.[3] The decreased energy intake in the obese mothers did not change the growth of their babies,[31] indicating that the diet restriction (energy) leads to weight loss among mothers, which neither changes the production of milk nor affects the growth of infants.[32,33]

In contrast to the findings mentioned earlier, there are studies that report the essential fatty acids such as omega-3 and omega-6 levels did not match with breast milk levels in spite of the positive correlation of the lipid composition with mother's nutrition.[22] Few other studies have demonstrated no correlations with maternal intake of fatty acids and breast milk,[34] nor with vegetarian and nonvegetarian food, referring to fat and micronutrients.[35] A study which reported no difference in the milk lipids compared to undernourished and the well-nourished mothers states that the long-term diet and body stores of the mother influence the lipids in the breast milk rather than the intestinal absorption of lipids.[36] It is also observed in few studies that prenatal administration of omega-3 fatty acids failed to demonstrate the favorable effect on neurodevelopment and cognitive outcome in the newborn.[37,38]

Dietary Protein and Quality of Breast Milk

Majority of the studies have shown that there is no relation with protein intake (animal protein and vegetarian diet) and breast milk content.[39-41] However, a study in the past has reported that milk samples of mothers who consumed more proteins in the diet showed significantly high levels of proteins.[42] It was also shown that the quality of breast milk may be associated with body composition of the mother rather than the diet.[43]

MICRONUTRIENTS/MINERALS

The maternal micronutrient intake is below the recommended values in >50% of cases.[44] It has been studied that some of the micronutrients in breast milk are influenced by mother's diet such as vitamins A, B_2, D, and E and niacin; although all micronutrients in breast milk may not reflect the maternal intake.[45] Vitamin E levels, especially in transitional milk, are reported to be more in mothers with high vitamin E intake and their serum levels.[46]

Group 1 nutrients (B vitamins) are known to be associated with maternal dietary intake[47] and vitamin B_6 levels are reported to vary with dietary intake during gestational period and postpartum phase,[48] while breast milk concentration of group 2 nutrients (folate, iron, zinc, copper, and calcium) are independent of maternal food intake.[3] Another study has reported that iodine in breast milk varies with maternal intake levels.[49] Iodine intake needs to be normal, although excess of iodine can also produce adverse effects in fetus, which is rare compared to iodine deficiency.[50]

Vitamin B_{12} deficiency in infants leads to serious neurological symptoms which can be prevented by vitamin supplementation in infants.[51] Breastfed infants rarely suffer from vitamin C deficiency irrespective of maternal diet or supplementation of vitamin to infants.[52] Vitamin D levels in the infant depends on the vitamin status of the mother and supplementation of vitamin D in mother is known to prevent deficiency in the baby.[53] In contrast to these reports, it is shown that most of the trace elements have not shown any association with maternal nutrition; however, manganese seems to be influenced by maternal intake.[54-56]

Although the quality of milk is not much affected by maternal diet,[57] there is growing evidence which suggests that infants do not escape the effect of nutrient deficiency in mother during pregnancy and lactation, especially referring to the critical nutrients such as DHA, choline, folate, iodine, and vitamin D.[58-61] Choline is known to have important role in memory.[62] Some of the essential and nonessential trace minerals found in breast milk could be the result of mother's diet and environment or could have been consumed as smoke.[63]

In short, the studies have shown inconsistent association between maternal diet and breast milk nutrients. Among all the nutrients, fatty acids in human milk seem to be sensitive to maternal diet and in turn influence the infant brain development.[64] In Indian scenario too, it is reported that breast milk composition remains relatively constant irrespective of mother's nutrition, which is similar to other previous study reports.[65] The colostrum milk also does not vary much between well-nourished and poorly nourished mothers; however, fat and protein content may reflect mother's nutritional status.[66] Maternal diet may have more effect on milk composition in later postpartum phase rather than initial period of lactation.[67] Mothers with optimal nutrition can make healthy babies;[68] hence, taking care of mother's nutrition will positively favor the infant's overall growth.[69] It is advisable to maintain good nutrition of mothers, especially in developing countries such as India.

Recent findings suggest exclusive breastfeeding for 6 months might also be associated with micronutrient deficiencies in infants, which has led to prescribing nutrient supplementation during infancy. However, this discussion needs to be supported by more research before concluding the insufficiency of exclusive breastfeeding.

The review analysis reinforce the finding that vitamins (B and D) and DHA concentrations are affected by maternal nutrition.[70] The dietary guidelines suggested especially for Indian community are to be considered for mothers and growing infants, which ensure regaining birth weight and optimal growth of a child at various phases to attain complete genetic potential.[71]

KEY MESSAGES

- The studies have shown inconsistent association between maternal diet and breast milk nutrients.
- Among all the nutrients, fatty acids in human milk seem to be sensitive to maternal diet and in turn influence the infant brain development.
- The fetal nervous system is very sensitive to the maternal dietary supply of essential fatty acids.
- The critical nutrients such as DHA, choline, folate, iodine, and vitamin D need to be optimally supplied in mother.
- Mothers with optimal nutrition can give birth to healthy babies.

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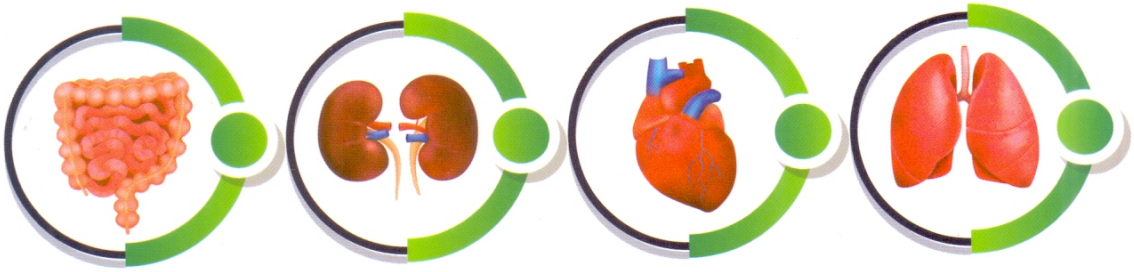

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CHAPTER

Subclinical Hypothyroidism: When to Treat?

Arathi Darshan

ABSTRACT

Subclinical hypothyroidism (SCH) is a common disorder characterized by elevated serum thyroid stimulating hormone (TSH) levels. This condition occurs in 3–8% of general population. The most important implication of SCH is high likelihood of progression to overt hypothyroidism. SCH is associated with adverse maternal and fetal outcomes that may improve with treatment. Current guidelines recommend, routine levothyroxine therapy for individuals with a persistent serum TSH more than 10 mU/L and individualized therapy for those with a TSH of <10 mU/L.

INTRODUCTION

Primary hypothyroidism can be classified as overt hypothyroidism when TSH values exceeds >10 mU/L (non pregnant individuals) and SCH when TSH values are between 4.5 and 10 mU/L.

Subclinical hypothyroidism (SCH), also called mild thyroid failure, is diagnosed when serum thyroid-stimulating hormone (TSH) is elevated in the presence of normal serum free thyroxine (T4) concentration. It is a common disorder in day-to-day practice. There is a higher prevalence of SCH in women, elderly individuals, and those with thyroid autoimmunity. Appropriate treatment of SCH may prevent its progression to overt hypothyroidism. SCH is associated with adverse cardiovascular events, adverse maternal and fetal outcomes, neuromuscular and cognitive abnormalities

Current guidelines recommend routine treatment with L-thyroxine for individuals with a persistent rise in serum TSH of >10 mU/L, in nonpregnant patients. Management of SCH with serum TSH values between 4 and 10 mU/L is controversial. Treatment in such patients, should be individualized depending on the age, clinical features, presence of goiter, presence of antibodies to thyroid peroxidase (TPO), pregnancy status, individuals with infertility and comorbid states like CVD.

DEFINITION OF SUBCLINICAL HYPOTHYROIDISM

Subclinical hypothyroidism is a condition in which there is a persistent elevation of TSH (12 weeks or longer) in the setting of a normal free T4/T4 concentration, which are repeatedly found within the reference interval.

DIAGNOSIS OF SCH?

By definition SCH is asymptomatic. SCH is primarily a laboratory diagnosis. The most accepted initial screening test for SCH and overt hypothyroidism is serum TSH. TSH levels typically range between 0.4–4 mU/L, according to American Thyroid Association (ATA).

The ATA recommends screening for hypothyroidism starting at the age of 35 years with repeat screening every 5 years.

Definition of Elevated Levels of Thyroid-stimulating Hormone?

Controversies exist on the upper limits of TSH. TSH values depend on age, gender, ethnicity, body mass index (BMI), and pregnancy status. In the United States National Health and Nutrition Examination Survey 111 (NHANES 111),

which enrolled 16,533 people without thyroid disease, the upper limit of TSH reference value was 4.2 mU/L. There is a physiological rise in TSH values in elderly individuals. The upper limit of TSH in individuals above 80 years of age is 6–8 mU/L. Age-specific reference ranges for TSH interpretation are recommended.

A transient rise in TSH should be ruled out, before the diagnosis of SCH. Repeat thyroid function test is recommended after 2 months, for the confirmation of diagnosis of SCH. Most common causes of transient elevation in TSH values are nocturnal measurement of TFT and physiological rise in TSH values with ageing.

Other causes are recovery phase of nonthyroid illness, severe obesity, medications like amiodarone and chronic lithium therapy, assay variability, presence of heterophile antibodies interfering with TSH assay, untreated Addison's disease, central hypothyroidism, and thyroid hormone resistance.

PREVALANCE OF SUBCLINICAL HYPOTHYROIDISM

Prevalence of SCH varies from 4 to 20% in general population. Higher prevalence is seen in women, older individuals and in individuals with suboptimal iodine status. However, the prevalence is determined by the upper limit of TSH values **(Table 1 and Box 1)**.

CLINICAL FEATURES OF SUBCLINICAL HYPOTHYROIDISM

About 70% of patients with SCH are asymptomatic. Symptoms may be vague and nonspecific. Symptoms are similar to overt hypothyroidism. Fatigue, weakness, cramps, weight gain, constipation, impaired concentration, depression, anxiety, and poor quality of life have been frequently reported. Fatigue is one of the symptoms which is considered as an indication to start treatment in SCH.

TABLE 1: Epidemiology of subclinical hypothyroidism (SCH) in India.

Reference	*n*	SCH (%)
Unnikrishnan et al. (2013)	5,370	8
Unnikrishnan et al. (2011)	971	9.4
Rohil et al. (2010)	1,741	2.6
Abraham et al. (2009)	505	9.5
Sahu et al. (2009)	633	6.4

CONSEQUENCES OF SUBCLINICAL HYPOTHYROIDISM

Progression to Overt Hypothyroidism

The natural history of SCH is that it can either progress to overt hypothyroidism or have a spontaneous recovery. The annual rate of progression to overt hypothyroidism is approximately 3–18%. The underlying disease and patient characteristics are the major factors determining the risk of conversion to overt hypothyroidism.

The risk factors for progression to overt hypothyroidism include older individuals, female gender, and elevated titers of thyroid peroxidase antibodies TPOAb.

The underlying disease may also determine the progression. Patients who have received radioiodine ablation for Graves' disease, history of external radiation therapy for non-thyroid malignancies and those who have received chronic lithium treatment are likely to convert to overt hypothyroidism

Spontaneous recovery and normalization of TSH are more likely to occur in patients with initial TSH values of <10 mU/L and with negative antithyroid antibodies. In a study of 422,242 individuals without a known thyroid disease, serum TSH was elevated (>5–10 mU/L) in 3% of individuals. During a 5-year follow up-period, TSH values became normal without treatment in 62% of patients, within the first 2 years of diagnosis.

BOX 1: Causes of subclinical hypothyroidism (SCH).

- Chronic autoimune thyroiditis
- Persistent TSH increase—in subacute thyroiditis, postpartum thyroiditis, painless thyroiditis
- Thyroid injury—partial thyroidectomy or other neck surgery, radioactive iodine therapy, external radiotherapy for head and neck
- Drugs impairing thyroid function—iodine and iodine-containing medications (amiodarone, radioactive contrast agents), lithium, cytokines (especially interferon alpha), sulfonamides, sulfonylureas
- Inadequate replacement therapy for overt hypothyroidism—inadequate dosage, noncompliance, drug interactions
- Thyroid infiltration—amyloidosis, sarcoidosis, Reidel's thyroiditis, AIDS
- Central hypothyroidism
- Toxic substances, industrial and environmental agents
- TSH receptor gene mutations

(AIDS: acquired immunodeficiency syndrome; TSH: thyroid-stimulating hormone)

Source: Biondi B, Cooper DS. The clinical significance of subclinical thyroid dysfunction. Endocr Rev. 2008;29(1):76-131.

Cardiovascular Disease

Meta-analysis of several prospective cohort studies have reported, a significant increase in the risk of coronary heart disease and heart failure in patients with overt hypothyroidism (Serum TSH >10 mU/L).

Large number of studies support an association between subclinical hypothyroidism and cardiovascular events like CVD and heart failure. This association was strongly seen in patients of less than 65 years of age.

Increased CV risk may be explained due to the presence of atherogenic lipid profile [elevated total cholesterol and low-density lipoprotein (LDL) cholesterol levels], higher systolic and diastolic blood pressure, and increased carotid intima-media thickness. The most consistent cardiac abnormality reported in SCH is impaired left ventricular (LV) diastolic function. In overt and SCH, there is reduced peripheral action of thyroid hormone, thus leading to altered intracellular calcium handling. This results in slowed myocardial relaxation and impaired LV filling. LV systolic dysfunction with exercise, impaired endothelial function, increased vascular tone at rest, increased systemic vascular resistance, and arterial stiffness has been reported.

Reproductive Abnormalities and Pregnancy Outcome

Thyroid screening is important in women with reproductive abnormalities. There is an increased risk of pregnancy loss in women with SCH. This risk is more in the presence of positive anti-TPO antibodies. Data from various observational studies have demonstrated improved fertility outcomes after initiation of treatment. 44–84% of women treated with levothyroxine successfully conceived after initiation of treatment.

Undetected SCH in pregnancy is a risk factor for poor pregnancy outcomes in mother and fetus. Spontaneous abortion, preeclampsia, gestational hypertension, gestational diabetes, premature birth, low birth weight, and poor developmental outcomes in offspring have been reported.

Nonalcoholic Fatty Liver Disease

Studies have shown an association between TSH levels and nonalcoholic fatty liver disease (NAFLD). 30–36% of individuals with SCH and overt hypothyroidism had ultrasonographic findings of NAFLD (vs. 20% of controls), 20–26% had abnormal liver enzymes, nonalcoholic steatohepatitis (NASH) was seen in 58%, and fibrosis was reported in 26% of patients.

Neuromuscular Dysfunction

Studies have reported fatigue, weakness, paresthesias, and cramps in patients with SCH, which can be reversed with L-thyroxine therapy.

Psychiatric and Cognitive Dysfunction

In a large population-based study, SCH was associated with depression, cognitive dysfunction, and bipolar disease.

WHEN TO TREAT SUBCLINICAL HYPOTHYROIDISM?

Treatment of SCH depends on the following factors:

- *TSH values:* <10 or >10 mU/L
- *Age of the patient:* >65 years or <65 years
- *Patient clinical profile:* Clinical features, presence of goiter, TPOAb, pregnancy or intention of pregnancy
- *Comorbidities:* Presence of CVD, heart failure, dyslipidemia, obesity

Recommendations Depending on Thyroid-stimulating Hormone Values

TSH > 10 mU/L (in Nonpregnant Patients)

There is a strong recommendation from American Thyroid Association and Endocrine society of India, to treat all patients of overt hypothyroidism with TSH values > 10 mU/L.

Recommendations Depending on Age

Elderly patients > 65 years of age with TSH value < 10 mU/L

Treatment of SCH in this group of patients is not recommended, due to the absence of benefit. Since there is a physiological rise of TSH with ageing, unintended overtreatment can lead to complications in elderly. Treatment in older patients is indicated only in those who have convincing symptoms of hypothyroidism.

For younger patients < 65 years of age with TSH < 10 mU/L

Treatment is recommended in younger individuals with TSH <10 mU/L, in view of increased CV mortality and an increase risk of progression to overt hypothyroidism **(Box 2)**.

MANAGEMENT OF SCH

Management of subclinical hypothyroidism is shown in **Flowchart 1**.

BOX 2: Factors favouring levothyroxine treatment for patients < 65 years of age with TSH levels of 5–10 mU/L.

- Degree of TSH raised (2 TSH levels >8 mU/L)
- Progressive TSH increase
- Presence of goiter
- Presence of antithyroid antibodies
- Therapeutic trial for clinical symptoms
- Patient preference
- Young age of the patient
- Cardiovascular risk factors or prevalent CHD
- Smoking
- Dyslipidemia
- Bipolar disorder, depression
- Pregnancy or intention of pregnancy
- Infertility, ovulatory dysfunction
- Childhood and adolescence

Source: Javed Z, Sathyapalan T. Levothyroxine treatment of mild subclinical hypothyroidism: a review of potential risks and benefits. Ther Adv Endocrinol Metab. 2016;7(1):12-23.

TREATMENT OF SUBCLINICAL HYPOTHYROIDISM

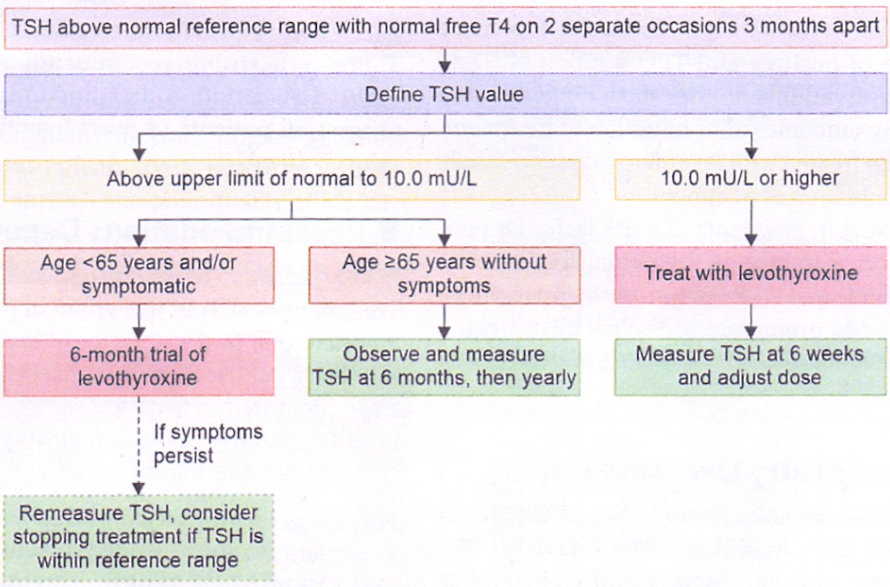
Before initiating the treatment of SCH with L-thyroxine, conditions causing transient and physiological rise in serum TSH should be ruled out. Confirmation with repeat testing of TSH and free T4 is recommended after 8–12 weeks, before initiating therapy. Therapy should not be delayed, when there are compelling indications of benefit of immediate therapy, like pregnancy or infertility.

Dosage

Factors to be considered before starting therapy are age of the patient, severity of the condition, and presence of comorbid conditions like CVD. Guidelines recommend initiating therapy with the lowest dose necessary to normalize serum TSH. Typically, 25–50 μg of L-thyroxine is recommended in patients with SCH. This approach is most appropriate to avoid overtreatment in elderly patients and with underlying CVD.

Goals of Therapy

The goal of therapy is to achieve euthyroid status, resolution of symptoms, and avoid over- or undertreatment. A TSH target of 0.5–2.5 U/L in young and middle-aged patients is recommended. In elderly patients beyond



FLOWCHART 1: Approach to management of subclinical hypothyroidism (SCH).
(TSH: thyroid-stimulating hormone)
Source: NICE 2019 Guidelines: Thyroid disease: Assessment and management.

65 years of age, a higher TSH target of 3–5 mU/L is recommended.

Monitoring of Therapy

Serum TSH should be repeated 8–12 weeks after initiation of therapy. If the target TSH is not achieved, the dose of L-thyroxine should be increased with a minimum dosage of 12.5–25 μg. If serum TSH is low, the dosage should be decreased accordingly. Once normal TSH values has been achieved, TSH should be repeated after 6 months and then annually.

For untreated patients of SCH who are not on treatment, repeat TSH is recommended after 6 months and thereafter annually.

SUBCLINICAL HYPOTHYROIDISM IN PREGNANCY

Diagnosis of SCH differs slightly in pregnant women. Diagnostic thresholds are different in pregnancy and trimester-specific references are recommended by the American Thyroid Association. The dosage of L-thyroxine in pregnant women is 1.2 μg/kg/day.

CONCLUSION

SCH is usually asymptomatic and is primarily a laboratory diagnosis. Transient rise in TSH levels should be ruled out, before initiating treatment. SCH is associated with increased risk of CV events, poor pregnancy outcomes and has a high risk of progression to overt hypothyroidism. Treatment is strongly recommended for all non-pregnant patients, with persistent elevation of TSH > 10 mU/L. However, routine treatment of patients with serum TSH between 4.5 and 10 mU/L remains controversial. In elderly individuals above 65–70 years of age with TSH < 10 mU/L, treatment is routinely not recommended. For individuals below 65 years of age with TSH < 10 mU/L, treatment decisions are based on individual patient profile and co-morbid states.

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Legal Issues in Pediatric Practice

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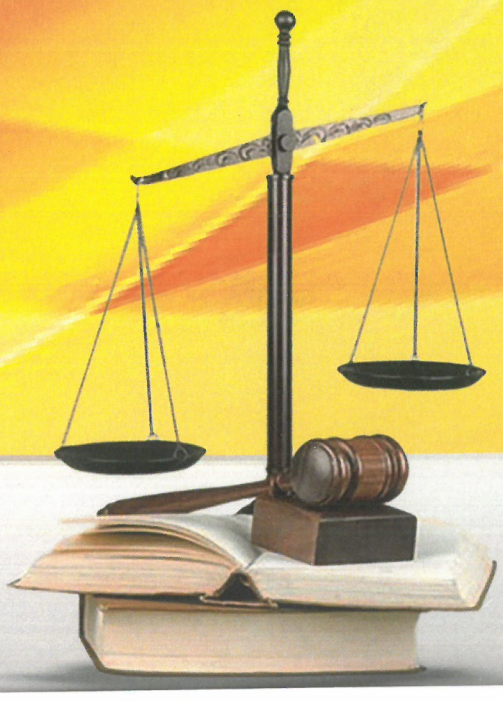

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Chapter

12

Insights into Pediatric Age, Role of Pediatricians, Qualification Aspects of Pediatric Care

Mahesh Baldwa, Namita Padvi, Varsha Baldwa, Dnyanesh DK

Ensure life worth living after the treatment of sick and serious is over

Key words: • Pediatric age • Child • Minor • Qualifications

Aim: *The aim of studying medicolegal issues in pediatric practice is to ensure that healthcare providers and institutions caring for children understand and adhere to legal and ethical principles, safeguarding the rights, safety, and well-being of pediatric patients while mitigating risks, maintaining standards of care, and addressing potential legal challenges.*

Objective: *The objectives of exploring medicolegal issues in pediatric practice are to educate healthcare professionals about the legal framework governing pediatric care, enhance their awareness of ethical dilemmas, facilitate compliance with legal requirements, promote patient advocacy, and ultimately, improve the quality of care provided to children while reducing legal liabilities and disputes.*

INTRODUCTION

Over the past five decades, the field of pediatric medical science has experienced continuous progress. Notable advancements in science and technology, increased longevity, reduced mortality and morbidity rates, and an overall enhancement in the quality of life mark some of the positive changes witnessed. This evolution has transformed the art of medicine into a comprehensive medical science.[1,2]

EXPLORING THE CONCEPT OF PEDIATRIC AGE

Defining the age of childhood or adolescence lacks a specific statutory or legal boundary. The term "child" is not explicitly defined in the constitution or the General Clauses Act and does not equate to "minor." Various Acts provide differing definitions. According to the Indian Majority Act, 1875,[3] a child is considered to be up to 18 years old, unless specific exceptional cases apply, in which case it becomes 21 years. The Children Act, 1960[4] defines a child as a boy below 16 years of age or a girl below 18 years. The Indian Academy of Pediatrics suggests that pediatricians should continue to care for and treat children up to the age of 18. The American Academy of Pediatrics, on the other hand, follows the guidelines for choosing pediatric experts for advisory panels, the US Department of Health and the Food and Drug Administration reference approximate age ranges for these phases of life, which consist of the following: (1) newborn up to 1 month, infancy, between 1 month and 2 years of age; (2) childhood, from 2 to 12 years of age; and (3) adolescence, from 12 to 21 years of age. [US Department of Health and Human Services; Food and Drug Administration. Guidance for Industry and FDA Staff: Pediatric Expertise for Advisory Panels. Rockville, MD: US Department of Health and Human Services, Food and Drug Administration, Center for Devices and Radiological Health; 2003. Available at: https://www.fda.gov/media/72451/download, accessed on 20.11.2023].[1,2]

Reading chapter 14 shall be helpful in understanding medico-legal concepts with respect to newborns and pre-matures.

UNDERSTANDING THE ROLE OF A PEDIATRICIAN

According to the World Health Organization (WHO), a physician is someone who has been duly admitted to a recognized medical school and has successfully completed the prescribed medical studies, obtaining the necessary qualifications for legally practicing medicine, which includes prevention, diagnosis, treatment, and rehabilitation, while exercising independent judgment to promote community and individual health. A specialist is defined as someone who specializes in a specific field of medicine. A Pediatrician, also known as a "Child Specialist," is a doctor who has specialized

in the care of children. In accordance with the National Medical Commission 2019,[5] approved by the Indian Medical Council (Professional Conduct, Etiquette, and Ethics) Regulation of April 2002, Clause 7.20 states that a physician cannot claim to be a specialist unless they possess specific qualifications in that particular branch. The Supreme Court has consistently emphasized that medical practitioners should practice within the scope of their qualifications and skills. so far MD pediatrics and DCH belonged to specialization of pediatric. But with advent and recognition of DM in Neonanatolgy and DM in Pediatric neurology several other options are available for doctorate of medicine in pediatric super-specialties which needs to be confirmed for recognition from National Medical Commission before undertaking studies in those super-specialties.[1,2]

KEY ASPECTS OF PEDIATRIC CARE

1. **History taking and examination:** The process begins with a thorough review of the patient's medical history. The physician must not only listen attentively to the patient's complaints but also inquire about any relevant positive or negative history related to those complaints, aiding in accurate diagnosis and ruling out common differential diagnoses. Diagnosis remains a dynamic and flexible concept. A well-documented history can often lead to a definitive diagnosis, whereas an incomplete or misleading history can lead to incorrect conclusions.
2. **Investigations:** When clinical diagnosis proves challenging, the use of diagnostic investigations becomes necessary. Tests that align with the presenting symptoms and signs should be recommended.
3. **Diagnosis:** In some cases, the symptoms and signs may overlap to the extent that clinical diagnosis becomes exceedingly difficult.
4. **Use of medicines/drugs:** Special care must be taken when prescribing drugs to children, considering their age. Some medications lack sufficient scientific trials or data for safe use in children. In exceptional circumstances, these drugs may be administered with strict precautions and monitoring, adhering to recommendations from reputable textbooks and scientific journals rather than relying solely on pharmaceutical company leaflets.
5. **Self-limiting diseases:** Diseases vary in terms of progression, from self-limiting to aggressive. Physicians must possess the ability to foresee common complications, diagnose them promptly, and provide appropriate treatment.
6. **Emergency situations:** Medical practice often involves handling emergency situations, where clinical features may not clearly indicate a specific diagnosis. Gathering a complete patient history may not be feasible, especially if the patient is unconscious or distressed, and there may be limited time for extensive investigations.
7. **Referrals:** When adequate facilities are not available, patients should be referred to well-equipped centers capable of providing the necessary care.
8. **Delegation of duties:** Physicians have the option to delegate their responsibilities to qualified and competent juniors, assistants, partners, nursing staff, or laboratory assistants.
9. **Delegation of duties:** A doctor can delegate his duties to a qualified and competent junior, assistant, partner, nursing staff or laboratory assistant.

About Cases Related to Qualifications

Poonam Verma vs Dr. Ashwin Patel, 1996 (2) CPJ 1 (SC)[6]

Dr. Ashwin Patel, BHMS, a Homeopath, prescribed Ampicillin and paracetamol for a viral fever. Subsequently, the patient's condition worsened, leading to hospitalization at a local nursing home and later transfer to Hinduja Hospital in Mumbai, where the patient tragically passed away due to diabetic keto-acidosis. AIIMS in Delhi opined that the administration of Ampicillin and Paracetamol was appropriate. However, the Supreme Court ruled that Dr. Ashwin Patel, not being qualified as an allopathic physician and lacking a background in pharmacology, was not authorized to prescribe allopathic medications. Consequently, the principle of negligence per se was applied, and Dr. Ashwin Patel had to pay a sum of 3,30,000 rupees.

MBBS DCH Can Practice Pediatrics and Neonatology

Dr. P. Madanmohan Rao vs Kharja Moizuddin, 2004 (7) CLD 313 (Andhra Pradesh SCDRC)[7]

"The treatment of newborn babies - Qualifications - OP-1, possessing a medical degree (MBBS) and OP-2, holding an MBBS degree along with a diploma in Pediatrics and a Fellowship in Neonatology, both duly registered with the Medical Council of India and the State Medical Council - The District Forum's decision to uphold the complaint, alleging a deficiency in service, based on the doctors' supposedly lacking qualifications, without any claims of service-related shortcomings in patient care or the proper treatment

procedures - Such a ruling is untenable as no evidence has been presented to demonstrate that the doctors were ineligible to practice pediatrics."

Numerous Cases Serve as Clear Reminders of Certain Fundamental Principles in the Medical Profession

1. It is imperative to operate within the boundaries of one's qualifications and competencies.
2. One should refrain from representing themselves to the public for practices they are not qualified to perform.
3. Practitioners should not engage in, or profess to be experts in, areas of medicine they have not formally studied during their medical education.

Legal Requirements for Medical Practice are Established through Various Legal Precedents

1. Medical professionals should possess a reasonable level of knowledge within their respective category of doctors, striking a balance between exceptionally high and unacceptably low competence. This principle was established in the case of Laxman Balkrishna Joshi versus Trimbak Bapu Godbole, 1969(1) SCR, 206.[7]
2. The adequacy of a medical professional's equipment and infrastructure should align with the types of cases they undertake. Typically, this is evaluated based on the equipment commonly possessed by their peers in the local area.
3. Medical practitioners are duty-bound to provide first aid to emergency patients, necessitating the availability of emergency medicines and injections as per the rule of law established in the case of Parmanand Katara versus Union of India, 1989, ACJ 1000, SC.[8]
4. While physicians are not expected to read every medical journal, they should refrain from persistently practicing outdated forms of treatment. This principle stems from the judgment in the case of Crawford versus Board of Governors of Charring Cross Hospital, 1953, Times 8th December, CA.[9]
5. Medical doctors should administer treatment to their patients with care and caution, selecting one of the recognized forms of treatment based on their own experience and expertise. This principle is exemplified by the decision in Bolam versus Friern Hospital Management Committee, 1957, 2 All ER 118 is still valid though in UK judgment in Montgomery v Lanarkshire Health Board [2015] UKSC 11 has rejected peer approved standard of medical care along with standards in Bolitho and Sidaway to patient centric logical , reasonable standard of medical care.[10]

SUMMARY AND CONCLUSIONS

Accusations of medical malpractice are a common occurrence in the lives of pediatricians. In the United States, nearly one-third of pediatricians face lawsuits, and while precise statistics are unavailable for India, it is frequently heard that pediatricians there also encounter legal challenges. Pediatricians should acknowledge this reality and proactively implement measures to decrease their susceptibility to legal action. It provides insights from past legal cases that can help pediatricians devise strategies to lower the risk of litigation and simplify the process of defending against claims with respect to qualification, knowledge, skill and experience.

Key Learning Points

1. Keep in mind that there is no legally defined age limit for children or adolescents.
2. Note that neither the constitution nor the General Clauses Act provides an exact definition for the term "Child."
3. Understand that "Child" is not synonymous with "minor."
4. Be aware that definitions may vary across different laws and regulations.
5. According to the Indian Majority Act, 1875, an adult is considered to be someone above 18 years old.
6. Follow the recommendation of the Indian Academy of Pediatrics, which suggests that pediatricians should continue to examine and treat individuals up to the age of 18.
7. Recognize that, according to the American Academy of Pediatrics: (1) newborn up to 1 month, infancy, between 1 month and 2 years of age; (2) childhood, from 2 to 12 years of age; and (3) adolescence, from 12 to 21 years of age.
8. Understand that a specialist is someone who delves deeply into a specific area of expertise. A pediatrician, or "Child Specialist," is a doctor who specializes in the care of children. According to the Indian Medical Council (Professional Conduct, Etiquette, and Ethics) Regulations of April 2002, as approved by the NMC - 2019, Clause 7.20 states that a physician should not claim to be a specialist unless they possess special qualifications in that field. While MBBS doctors can practice pediatrics alongside general medicine, they should refrain from claiming to be pediatricians. This issue falls into a gray area and requires clarification from the National Medical Commission - 2019, as there is no established distinction between MBBS

practitioners treating pediatric patients and those with MD, DNB, DCH qualifications treating pediatric patients. Both are legally allowed to provide care to pediatric patients.DM super-specilities in neonatology and neurology and other sub-specialites are adding confusion to area of pediatric practice domins, as to who should do what?

Take Home Messages

The Indian Academy of Pediatrics suggests that pediatricians should continue to care for and treat children up to the age of 18.

Must Avoid Things

According to the Indian Medical Council (Professional Conduct, Etiquette, and Ethics) Regulations of April 2002, as approved by the NMC - 2019, Clause 7.20 states that a physician should not claim to be a specialist unless they possess special qualifications in that field.

Do Not Do

Physicians are not expected to read every medical journal; they should refrain from persistently practicing outdated forms of treatment.

Warnings

Don't use SMC and NMC unrecognized qualifications as suffix even when they are connected to specializations in child health.

Messages which the Reader must be Aware

To the American Academy of Pediatrics: (1) newborn up to 1 month, infancy, between 1 month and 2 years of age; (2) childhood, from 2 to 12 years of age; and (3) adolescence, from 12 to 21 years of age.

Must do Thing

A pediatrician, or "Child Specialist," is a doctor who specializes in the care of children.

Medical Negligence Pearls

Pediatricians are medical doctors specializing in the care of infants, children, and adolescents.

They play a crucial role in promoting and maintaining the health of children, monitoring their growth and development, and addressing any medical concerns.

Only One Fact to Remember

According to the Indian Majority Act, 1875, an adult is considered to be someone above 18 years old.

MCQs

1. Supreme Court judgment in a case of Poonam Verma vs Dr. Ashwin Patel is about:
   a. Crosspathy practice
   b. Allopathy practice
   c. Quackery
   d. It is wrong for homepath to prescribe allopathic medicine

   Ans. d
2. Decided legal cases giving loud and clear message that pediatrician should work:
   a. Work within the scope of one's qualification and skills
   b. Do not hold out to public at large, what you cannot do and for what, one is not qualified
   c. Do not practice and hold out to people at large, what you have not undertaken to study while getting medical qualifications
   d. All of the above

   Ans. d

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Baldwa's

Legal Issues in Pediatric Practice

There are experiences ever-evolving new legal challenges targeted towards practicing pediatric specialists. Legal concerns in medical practice catch pediatricians off guard with each passing day. The utilization of seemingly innocuous terms like iatrogenic, known cases of, known complications and known as causing death or disability is equated with alleged medical malpractice, among a host of other accusations. People at large have become aware that pediatric medical care, vaccinations and allied services fall under the purview of the Consumer Protection Act 2019 and National Medical Commission (NMC) 2019. New code of conduct was brought by NMC on 02.08.2023 and put in abeyance on 23.08.2023, thus a status quo for generic prescriptions and pharma sponsorship continues.

Chimera of s. 106(1) of Bharatiya Nyaya Sanhita 2023 is apparently harsher than IPC 304A, a way away from complete decriminalization as promised on December 20,2023, by Union Home Minister. IPC-1860 is set to be replaced by New Bharatiya Nyaya Sanhita 2023(BNS), CrPC1973 by Bharatiya Nagarik Suraksha Sanhita Act, 2023 (BNSS), IEA1872 by Bharatiya Sakshya Adhiniyam 2023, so in future please refer to corresponding section before studying decided case laws.

Neither during medical undergraduate nor postgraduate medical training adequately prepares pediatricians for real-world scenarios of medico-legal importance, including allegations of medical malpractice, service deficiencies, and unfair trade practices. For the first time, pediatricians are recognizing the importance of smart communication of material risks and their documentation as to what to convey and what not to convey, the significance of counseling, forewarning, risk-disclosures, guarantees, and warranties, all of which contribute in preparing proper informed consent/contract for medical services. This book highlights the significance of why, when, how, where and what to document on OPD, IPD transfer, and discharge papers as rebuttable evidence through the proper preservation of medical records.

The distinguished authors have exerted every effort to disseminate their knowledge, experience, and wisdom to the readers by scrutinizing numerous significant judicial decisions involving alleged medical malpractice, deficiency of services, and unfair trade practices. References in the book are included at the point of description. Its objective is to make medical practice legally safe rendered ethically, with peace of mind, which is reassuring to both the patients and the doctors, and less stressful by addressing upfront numerous legal concerns related to medical practice which help in preventing litigations in general.

This book follows a reader-friendly format encompassing all practical and relevant topics. Each chapter has Aim, Objective, Summary, Key learning points, and one liner medico-legal wisdoms to aid in the retention of crucial legal topics relevant to medical practice.

The book makes an ideal reading for all practicing pediatricians in terms of the preventive aspects, legal problems in medical practice, as well as those confronting litigation. The book will prove valuable to all pediatricians, members of judiciary and concerned law-enforcing agencies, hospital management officials, officers of health insurance companies, and practicing lawyers handling matters related to medical negligence litigation.

Baldwa's Dictum: *Better qualified and skillful doctors take higher risks in medical field and get prosecuted*

Editors

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Art and Science of Breastfeeding & Beyond

Durgappa H
Anita Nyamagoudar
CR Banapurmath

Foreword
VD Patil

JAYPEE

CHAPTER 37

Infant Milk Substitute Act

Dhyanesh DK, Asha Doddamane Benakappa, Hari Dattatreya M

"Ignorantiajuris non excusat-Ignorance of law is not an excuse".[1]

INTRODUCTION

Case scenario 1: In a mother and child hospital, a mother of a term, healthy baby complained of inadequate milk secretion because of a sore nipple. The pediatrician prescribed formula milk without addressing the mother's issue. Is it a violation of the IMS (Infant Milk Substitutes) act?

Case scenario 2: In a maternity hospital, at the time of discharge, all the mothers were given infant formula milk as "TONIC" to their babies, as the formula milk company has promised a foreign tour to the doctor. Is it a violation of the IMS act?

In both cases, the answer is YES. "No person shall take part in the promotion of infant milk substitutes or infant foods". It is a clear violation of the IMS act and a punishable offense.

HISTORY OF IMS ACT

"Breastfeeding was the best, is the best, and will remain the best". As far as infant feeding is concerned, breastfeeding has been a part of our culture since ancient times, but with progressive transition, breastfeeding practices have gradually gone downhill.[2] Globally, it is estimated that 1.5 million babies die each year, because they are not adequately breastfed.[3] The International Code of Marketing of Breast Milk Substitutes was adopted by the World Health Assembly in 1981 to promote breastfeeding and to regulate the marketing of breast-milk substitutes and feeding bottles. In India, nearly one million children die each year because of improper feeding practices. The government of India recognized this code and adopted the Indian National Code for the protection and promotion of breastfeeding in December 1983. The international code is mainly regulatory. It regulates the promotion, sale, and marketing of teats, bottles, milk substitutes, and baby foods. The code insists that companies provide scientific unbiased and factual information about infant feeding. It does not prescribe any punishment to the offenders but gives power to the individual state government to frame their own specific rules to tackle the menace of artificial feeding. Based on the recommendation of "The WHA" (*World Health Assembly*), Indian government enacted the Infant Milk Substitute, Feeding Bottles, and Infant Foods (Regulation, Promotion, Supply, and Distribution) act 1992 (IMS act), which came into force on August

1st, 1993. It was further amended in 2003 to strengthen certain provisions and close any loopholes infant formula companies had found.[4]

OBJECTIVES OF THE IMS ACT

- To protect breastfeeding from commercial promotion and thus prevents malnutrition and deaths in infants and children.
- The IMS act regulates the marketing strategies of baby food manufacturers.[5]

IMPORTANT PROVISIONS OF THE IMS ACT[4]

Section 2(f) defines what is *infant food*: Infant food is any food (by whatever name called) being marketed or otherwise represented as a complement to the mother's milk to meet the growing nutrition needs of the infant after the age of 6 months and up to the age of 2 years.

Section 2(g) defines infant milk substitute: Infant milk substitute means any food being marketed or otherwise represented as a partial or total replacement for a mother's milk for an infant up to the age of 2 years.

Section 3: This is a very important section that deals with certain provisions about infant milk substitutes, feeding bottles, and infant foods.

- No person can advertise or take part in the publication of any advertisement for the distribution, sale, or supply of infant milk substitutes or feeding bottles, or infant foods.
- No person shall give an impression or create a belief in any manner that feeding of infant milk substitutes and infant foods are equivalent to or better than mother's milk.
- No person shall take part in the promotion of infant milk substitutes, feeding bottles, or infant foods.

Section 4: Deals with provisions about incentives for the use or sale of infant milk substitutes or feeding bottles or infant foods.

- No person can supply or distribute samples of infant milk substitutes or feeding bottles or infant foods or gifts of utensils or other articles.
- No person shall contact any pregnant woman or mother of an infant.
- No person shall offer the inducement of any other kind to promote the use or sale of infant milk substitutes or feeding bottles or infant foods.

Section 5 of the act states about the donation of infant products:

- No person shall donate or distribute infant milk substitutes or feeding bottles or infant foods to any person except to an orphanage.
- No person shall donate or distribute any informational or educational equipment or material relating to infant milk substitutes or feeding bottles or infant foods. But donation or distribution through the healthcare system under prescribed conditions and restrictions can be made.

Section 6: This mentions what information and labels should be there on containers of infant milk substitutes or infant foods. Every container thereof or any label affixed thereto indicates in a clear conspicuous and easily readable and understandable manner, the words "Important notice" in capital letters in such language as may be prescribed and indicating there under the following particulars in the same language.

- A statement "mother's milk is best for your baby" in capital letters.
- A statement that infant milk substitutes or infant food should be used only on the advice of a health worker as to the need for its use and the proper method of its use.

- A warning that infant milk substitutes or infant food are not the sole sources of nourishment for an infant.
- The instruction for its appropriate preparation and warning against the health hazards of its inappropriate preparation.
- No container shall have a picture of an infant or woman or both.
- No container shall have pictures or other graphic materials or phrases designed to increase the sale ability of infant milk substitutes or infant food.
- No container shall use on it the word "humanized" or "maternalized" or any other similar words.

Section 7: States that educational and other materials relating to the feeding of infants contain certain particulars, such as:

- The benefits and superiority of breastfeeding.
- The preparation for, and the continuance of breastfeeding.
- The harmful effects on breastfeeding due to the partial adoption of bottle-feeding.
- The difficulties in reverting to breastfeeding infants after a period of feeding by infant milk substitute.
- The financial and social implications of making use of infant milk substitutes and feeding bottles.
- The health hazards of improper use of infant milk substitutes and feeding bottles.

Section 8: Mentions the responsibility of the healthcare system.

- No person can use any healthcare system or the display of placards or posters relating to or for the distribution of materials to promote the use or sale of infant milk substitutes or feeding bottles or infant foods.
- No person who produces, supplies, distributes, or sells infant milk substitutes or feeding bottles or infant food can make any payment to any person working in the healthcare system to promote the use or sale of such substitutes or bottles or food.
- No person except a health worker can demonstrate feeding with infant milk substitutes or infant foods to the mother of an infant or any member of her family.
- No person except an institution or organization engaged in the healthcare system can distribute infant milk substitutes or feeding bottles to a mother who cannot resort to breastfeeding and who cannot afford to purchase infant milk substitutes or feeding bottles.

Section 9: This section of the IMS act is a very important section that deals with inducement to a health worker for promoting the use of infant milk substitutes.

- No person who produces, supplies, distributes, or sells infant milk substitutes or feeding bottles, or infant food shall offer or give directly or indirectly any financial inducements or gifts to a health worker or any member of his family to promote the use of such substitutes or bottles or foods.
- No producer, supplier, or distributor shall offer or give any contribution or pecuniary benefit to a health worker or any association of health workers, including funding of seminar meetings, conferences, educational courses, contests, fellowships, research work, or sponsorship.

Section 11: Mentions standards of infant milk substitute feeding bottles or infant foods and should be as for the standards specified under the Food Safety and Standards Act, 2006.

Section 13: Gives power to any food safety officer or authorized officer to seize infant milk substitutes or feeding bottles or infant

food if the provisions of this act have been or are being contravened. And liable for confiscation as per Section 14.

PUNISHMENT FOR VIOLATING IMS ACT

Section 20 of the IMS act mentions *Punishment* and *Penalty*. If there is a violation of provisions of Sections 3, 4, 5, 7, 8, 9, 10 and rules made under Section 26 of the Act shall be punishable with *Imprisonment* up to 3 years or with *fine* up to 5,000 rupees or with *both*. When an offense under this act has been committed by a company shall be liable to be proceeded against and punished accordingly as per *Section 22*.

IMPLEMENTATION AND MONITORING OF THE IMS ACT

The monitoring of the IMS act is undertaken by four nongovernmental organizations (NGOs), food safety officials, and other government officials authorized by the government as specified in Section 21(1). The Breastfeeding Promotion Network of India (BPNI) has been widely involved and successful in monitoring the IMS act. It conducts events to create awareness of infant formula, develop monitoring tools, regularly monitors product labeling and promotional activities of the infant formula companies, timely report to the ministry of women and child development, and files cases if violations of the law.[6] Exclusive breastfeeding rates have increased from 36.8% in 2000 to 58.3% in 2005 to 64.9% in 2014 compared with the Global average of 38% due to promotional, educational, and training activities regarding infant and young child feeding and also due to enforcement of legislation such as IMS act.[7]

ACTIONS ARE TAKEN FOR VIOLATING IMS ACT

Breastfeeding Promotion Network of India has a surplus of reports and letters regarding the success of monitoring the IMS act. (1) In 2010, an annual conference for Parenteral Nutrition and Enteral Nutrition sponsored by Nestle Nutrition Institute was canceled by the organizer when they received letters from BPNI and the Ministry of Health and Family Welfare. (2) In 2012, the regional Food and Drug Administration raided Nestle factory, which was using inappropriate graphics. Nestle was fined after the event. (3) In February 2016, the Indian Journal of Pediatrics blocked the circulation of their February issue, which contained infant formula advertisement.[8,9]

LIMITATIONS OF THE IMS ACT

1. Rules and regulations regarding infant food advertisements have many let-out clauses as compared to that for infant milk substitutes and feeding bottles.
2. There are no strict rules to address the issue of sponsoring scientific sessions, inducements, and other gifts by infant food manufacturers.
3. However, there are restrictions for infant food companies and health workers, but the chemists have been spared from many provisions of the act. The chemists continue to advertise and promote baby food products through the shops.
4. There is an acute need for prohibition and monitoring, promotion, and sales of IMS products, especially in e-marketing.[10]

STRATEGIES FOR THE EFFECTIVE IMPLEMENTATION OF THE ACT

Even though the government has enacted and passed the act, things are not working out as they expected. To defeat the aim of the act,

the manufacturers always look for loopholes in the law that are used by them for their benefit.

ROLE OF PARENTS

To make the act work, the parents have to play a very important role. The parents should know that exclusive breastfeeding till 6 months is a must for every child; it is the right of every child. After 6 months if the child is started on complementary feeds and accepting cereals, pulses, vegetables, fruits, etc. then *artificial food* is not at all essential. Every relative including the husband must help the mother in breastfeeding.[2,9]

ROLE OF NONGOVERNMENTAL ORGANIZATIONS

Various voluntary organizations like BPNI and the Association for Consumer Action on Safety and Health have already taken up the cause of fighting the menace of commercial foods by filing criminal cases against companies violating the IMS act. Because of this in 1996, Johnson and Johnson decided to stop marketing feeding bottles in India. These organizations also booked criminal prosecution against Nestle for violating the act. They are authorized by the Government of India.[11,12] These NGOs still need to be more vigilant for the effective implementation of the IMS act.

ROLE OF HEALTH WORKERS

The health workers along with relatives must counsel and motivate the mother and build her confidence in removing various misconceptions related to breastfeeding.[2,13] Health education regarding the advantages of breastfeeding and the disadvantages of commercial food must be imparted to the mother and her relatives. Human lactation management training workshops and the Baby Friendly Hospital Initiative movement help in such education. All of us who are working for the betterment of child health must take a pledge to protect support and promote breastfeeding.

ROLE OF PROFESSIONAL BODIES

Professional bodies should develop Joint Action Plan for promoting, supporting, and protecting breastfeeding and improving awareness about the IMS act. Professional bodies like Indian Academy of Pediatrics (IAP), Federation of Obstetric and Gynaecological Societies of India (FOGSI), Indian Medical Association (IMA), Human Milk Banking Association (HMBA), and others can provide training platforms. These bodies also provide an internal platform for anonymous reporting of violations of the IMS act by its members.[6]

KEY MESSAGES

- The IMS act was enacted to promote, protect, and support breastfeeding.
- Rational and judicious use of infant milk substitutes is not banned under the IMS act.
- The aim is to prevent indiscriminate, unscientific, and overuse of artificial milk.
- It is our moral responsibility to monitor the violations of the act and report them to the appropriate authorities.
- Proper and optimal nutrition in infancy is one of the major strategies to prevent malnutrition-related mortality and morbidity in our future generation.[6]
- Save IMS Act and Save the Child.

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Art and Science of
Breastfeeding & Beyond

"Breastfeeding is the essence of life to the mother and baby." It lays a good foundation for a long quality of life. It is also the right of the mother and baby. Hence, it is essential to ensure a conducive environment for the mother and baby for the establishment of successful lactation.

Though most women wish to breastfeed their infants, they often face difficulties at the initiation and maintenance of breastfeeding. Therefore, they need to be provided with skilled practical assistance and support. There is a huge knowledge gap among the healthcare professionals regarding optimal infant and young child feeding practice.

This book provides comprehensive state-of-the-art information on optimal infant and young child feeding including the basics of human lactation, problems of breastfeeding and solutions, breastfeeding in special circumstances, research in lactation and ways to enhance breastfeeding rates across the nations.

This book is intended to empower healthcare professionals, medical and nursing students, and breastfeeding support groups to improve their knowledge and wisdom which in-turn is hoped to aid in enabling breastfeeding women.

This book is reader-friendly and hopes to help healthcare professionals, healthcare workers, medical and nursing students, practitioners, mother support groups, and the public at large.

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Medical Negligence in Pediatrics

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Foreword
GV Basavaraja

JAYPEE

CHAPTER 3

Medical Documentation: The Only Defense for Doctors

Dnyanesh DK, Suma Dnyanesh

Court always says—"If it is not documented, then it did not happen"!

Keywords: Documentation, EHR systems, Expert testimony

Aim: Doctors must be ready to produce written documents which should be their ultimate defense for medical negligence is documentation in court of law.

Objective: Doctors are best givers of medical care but are lazy to document that care on paper. This is to wake up entire medical fraternity to shed lethargy and start documenting whatever good care they give to their patient's.

INTRODUCTION

Medical documentation is an essential component of modern healthcare practice. Beyond its role in patient care, medical documentation holds immense medicolegal significance. A medical document is a document containing the chronological account of patient's examination and treatment.[1] Medical documentation is the lifeblood of the healthcare industry, serving as both a record of patient care and a crucial defense for doctors. Medical evidence comprises a good proportion of civil and criminal law suits brought to trial in legal proceedings. Therefore the documents which are properly stored can become protective armors for doctors against the fiery darts of litigation in a court of law. This chapter explores the multifaceted world of medical documentation, delving into its importance in legal matters and healthcare systems.

THE ESSENCE OF MEDICAL DOCUMENTATION

Medical documentation is comprehensive repositories of patient information, encompassing a diverse range of data such as patient histories, diagnoses, treatment plans, test results, medication logs, surgical notes, and informed consent records.[2] They serve several pivotal functions:

- *Patient care continuity:* Medical documentation ensure the continuity of patient care. They provide a historical account of a patient's health, helping healthcare providers make informed decisions.
- *Adherence to standards:* Medical documentation reflect adherence to established medical standards and guidelines. They serve as a benchmark for evaluating the quality of healthcare provided.
- *Informed consent:* Proper documentation of informed consent is pivotal in

protecting both patient's rights and healthcare providers' interests.

- *Communication tool:* Medical documentation facilitate effective communication among healthcare professionals, reducing the risk of misunderstandings and errors.

MEDICOLEGAL IMPORTANCE OF MEDICAL DOCUMENTATION

Medical documentation plays a central role in medicolegal matters, encompassing areas such as:

- *Evidence in legal proceedings:* In legal disputes, medical documents serve as primary sources of evidence. They provide a detailed account of patient care and can significantly influence court decisions.
- *Standard of care:* Adherence to the standard of care is a crucial element in medical malpractice cases. Properly maintained documents can establish whether healthcare providers followed established protocols.
- *Informed consent documentation:* The absence of well-documented informed consent can expose healthcare providers to legal liabilities. Medical documents are instrumental in substantiating the consent process.
- *Chronology of events:* Medical documentation create a chronological timeline of a patient's medical journey. This timeline is essential for determining the sequence of events and assessing whether negligence occurred.

THE LEGAL IMPORTANCE OF MEDICAL DOCUMENTATION: A GLOBAL PERSPECTIVE

The medicolegal importance of medical documentation extends worldwide, with numerous legal systems recognizing its significance. Some prominent examples include:

- *United States:* In the United States, medical documents are governed by the Health Insurance Portability and Accountability Act (HIPAA). HIPAA not only regulates the confidentiality and security of medical documents but also emphasizes their legal importance in medical disputes. Courts often rely on medical documents to establish standards of care and evaluate the merits of medical negligence claims.
- *United Kingdom:* In the United Kingdom, the General Medical Council (GMC) issues guidance on documentation for healthcare professionals. Failure to maintain accurate and comprehensive documents can result in disciplinary action. In legal cases, medical documents are pivotal in establishing whether healthcare providers met their professional obligations.
- *Australia:* Australia's healthcare system emphasizes the importance of proper medical documentation. In legal proceedings, medical documents serve as primary evidence, shaping the outcomes of medical negligence claims. The Australian Medical Association (AMA) provides guidelines for maintaining quality medical documents.
- *Canada:* Canada recognizes the legal importance of medical documentation, and healthcare providers are expected to maintain accurate and complete documents. In medical malpractice cases, courts rely on these documents to assess the standard of care and determine liability.

CASE LAWS DEMONSTRATING THE MEDICOLEGAL IMPORTANCE OF MEDICAL DOCUMENTATION

White versus Brown (1981)—United States

In this landmark case, the court emphasized the crucial role of medical documents in medical malpractice claims. The plaintiff alleged negligence in the administration of anesthesia. The court relied heavily on medical documents to assess the standard of care and determine whether negligence had occurred.

Bolam versus Friern Hospital Management Committee[3] (1957)—United Kingdom

The Bolam test, established in this case, is widely used to assess whether a healthcare provider has met the standard of care. Properly maintained medical documents that reflect adherence to established medical practices can support a healthcare provider's defense in cases where the Bolam test is applied.

Rogers versus Whitaker (1992)—Australia[4]

This Australian case highlighted the importance of informed consent documentation. The court ruled in favor of the plaintiff, emphasizing that healthcare providers must provide comprehensive information to patient's and document the informed consent process adequately.

Snell versus Farrell[5] (1990)—Canada

In this Canadian case, medical documents played a pivotal role in assessing the standard of care. The court used the documents to determine whether the healthcare provider had followed established protocols, ultimately influencing the legal outcome.

Legal Importance of Medical Documentation—Indian Perspective

In the Indian healthcare landscape, comprehensive and accurate medical documentation serves as the primary line of defense for doctors facing allegations of malpractice and negligence.

CASE LAWS DEMONSTRATING THE ROLE OF DOCUMENTATION IN DEFENSE FOR DOCTORS IN INDIA

Jacob Mathew versus State of Punjab[6] (2005)

In this landmark case, the Supreme Court of India underscored the pivotal role of comprehensive medical documents. The court held that well-maintained documents provide essential insights into the treatment process and can protect healthcare providers from unfounded allegations of negligence. The case set a precedent for the importance of medical documentation in establishing the standard of care.

Samira Kohli versus Dr Prabha Manchanda[7] (2008)

This case reaffirmed the significance of informed consent documentation. The Supreme Court emphasized that consent must be properly documented. Failure to do so can expose healthcare providers to legal liabilities, even if the treatment was medically sound. This decision reinforced the importance of meticulous documentation of patient consent.

Martin F D'Souza versus Mohd. Ishfaq[8] (2009)

The court in this case stressed the importance of clear communication among healthcare professionals, which can be achieved through proper documentation. Effective communication can prevent misunderstandings and reduce the risk of medical malpractice claims. This case illustrates how proper documentation can foster a collaborative healthcare environment.

Malay Kumar Ganguly versus Sukumar Mukherjee[9] (2009)

This case underlines how medical documentation can serve as crucial evidence in court. The court heavily relied on medical records to determine whether negligence had occurred. The meticulously maintained records played a decisive role in shaping the legal outcome, showcasing the power of comprehensive documentation.

Maharaja Agrasen Hospital versus Rishabh Sharma[10] (2020)

Brief facts: Complainant, Pooja Sharma, mother of Master Rishabh Sharma, preterm child admitted in Maharaja Agrasen Hospital for preterm care nearly for 5 weeks. Doctors did not carry out retinopathy of prematurity (ROP) screening much later mother noticed baby's abnormal visual response. Eye checkup then showed total retinal detachment. Parents approached National Consumer Disputes Redressal Commission (NCDRC) for medical negligence. The hospital and its doctors denied allegation of negligence, claimed ROP screening was carried out and it had not revealed any problems. The NCDRC appointed expert medical board from AIIMS. Report said no negligence. The NCDRC in its order said that "we are not convinced whether the ROP screening was done by the ophthalmologist. The progress sheet is devoid of details about ROP examination, viz., who performed it, the method, instruments used, and drugs used during ROP testing. The discharge summary also has not mentioned ROP advice. Thus, "no record means, it was not done". Both the parties aggrieved by the NCDRC judgment, appealed to honorable Supreme Court. Honorable Supreme Court upheld the order of National Commission and awarded INR 76 lakh compensation.

CHALLENGES IN MEDICAL DOCUMENTATION

While medical documentation is undeniably crucial, several challenges persist:

- *Time constraints:* Healthcare providers often face time constraints, making it challenging to maintain meticulous records.
- *Electronic health records (EHRs):* While EHRs offer convenience, they come with their own set of challenges, including data security concerns and potential inaccuracies.
- *Interoperability:* Ensuring seamless sharing of electronic records across healthcare institutions remains a challenge, potentially hindering patient care continuity.
- *Legibility and completeness:* Illegible or incomplete entries in medical records can undermine their legal value.

ERRORS IN MEDICAL DOCUMENTATION

Errors in medical documentation can have consequences for patient care and safety.

Some common types of errors in medical documentation include:

- *Incomplete records:* Missing or incomplete information can lead to misdiagnosis or inappropriate treatment. This may include omitting essential patient history, medications, or test results.
- *Illegible handwriting:* Poor handwriting can result in misinterpretation of notes and medication errors. Many healthcare facilities have transitioned to EHRs to address this issue.
- *Incorrect information:* Entering incorrect information, such as patient demographics, can lead to confusion and potential harm. Verifying patient identity is crucial.
- *Abbreviation errors:* Using abbreviations without proper context can be confusing and lead to medication errors. There are standardized lists of medical abbreviations to reduce this risk.
- *Lack of date and time:* Failing to timestamp entries can make it challenging to track the timeline of a patient's condition and treatment.
- *Failure to document changes:* Failing to document changes in a patient's condition or treatment plan can result in miscommunication among healthcare providers.
- *Copy-paste errors:* Reusing text from previous notes without updating or verifying its accuracy can lead to misinformation.
- *Disorganization:* Poorly organized documentation can make it difficult for healthcare providers to find essential information quickly.
- *Privacy violations:* Unauthorized access to or disclosure of patient information violates privacy regulations and can lead to legal and ethical issues.

WHEN MEDICAL DOCUMENTATION IS VIABLE IN COURT OF LAW AND WHEN IT IS INVALID?

Medical documentation can be critical evidence in a court of law, especially in medical malpractice cases, personal injury claims, and other legal disputes involving healthcare. Whether medical documentation is considered viable or invalid in court depends on several factors:

- *Legibility and accuracy:* Viable medical documentation must be legible and accurate. Illegible or erroneous records can be challenged and may be considered invalid.
- *Timeliness:* Timely documentation is crucial. Courts may question the credibility of medical records if there are unexplained delays in recording information.
- *Authentication:* Medical records should be properly authenticated to ensure their validity. This often involves the signature of the healthcare provider or an authorized staff member.
- *Chain of custody:* In cases involving laboratory test results or medical imaging, maintaining a secure chain of custody is essential to ensure the integrity of the evidence.
- *Compliance with standards:* The documentation should adhere to established standards and protocols. Deviations from these standards may raise doubts about the validity of the records.
- *Documentation of informed consent:* In situations where medical procedures or treatments were performed, documentation of informed consent is vital. Patient's should be adequately informed about the risks and benefits before giving consent.

- *Hearsay:* Courts generally do not accept hearsay evidence, which includes second-hand accounts or statements. Medical documentation should primarily contain firsthand observations and assessments.
- *Patient privacy:* Healthcare providers must comply with patient privacy laws. Unauthorized disclosure of patient information can render documentation invalid.
- *Retrospective alterations:* Any alterations or additions to medical records should be properly documented, dated, and explained. Retrospective changes without clear justification can damage the credibility of the documentation.
- *Documentation by qualified personnel:* Medical records should be generated by qualified healthcare professionals or authorized personnel. Notes from unqualified individuals may not hold up in court.
- *Expert testimony:* In many cases, expert witnesses, such as medical professionals, are called upon to interpret and testify about the validity and significance of medical records.
- *Preservation of records:* Medical records should be preserved according to legal requirements and institutional policies to prevent loss or tampering.

In summary, viable medical documentation in a court of law is typically:

- Legible, accurate, and timely
- Properly authenticated and compliant with standards
- Generated by qualified personnel
- Free from unauthorized alterations or additions
- Protected patient privacy
- Supported by expert testimony when necessary.

Invalid medical documentation often involves issues with legibility, accuracy, authenticity, or compliance with legal and ethical standards. It is important for healthcare providers and institutions to maintain meticulous and legally compliant medical records to ensure their credibility and usefulness in legal proceedings.

DOCUMENTATION DURING MEDICAL EMERGENCY[11]

It should be done as comprehensively and accurately as possible, considering the urgency of the situation. Here is a general guideline on how and when to document a medical emergency:

- *Patient safety first:* In any medical emergency, the immediate priority is patient safety and providing necessary medical care. Ensure the patient receives appropriate and timely interventions without unnecessary delays.
- *Initial assessment:* Begin documenting as soon as possible after ensuring the patient's initial stabilization. This might involve assessing vital signs, starting treatment, or performing lifesaving interventions.
- *Continual updates:* Continuously document changes in the patient's condition, interventions performed, medications administered, and the response to treatments. Document with time stamps to create a chronological record.
- *Conciseness and clarity:* In emergency situations, brevity and clarity are essential. Use concise language to describe observations, interventions, and responses. Avoid unnecessary details that do not directly impact patient care.
- *Standardized forms or electronic records:* Use standardized forms or her,

if available. These systems often have predefined templates for emergencies, making documentation more structured and efficient.

- *Timestamps:* Always include timestamps for entries to establish a clear timeline of events. This is crucial for assessing the patient's progress.
- *Patient information:* Clearly document the patient's name, date of birth, and other identifying information. This ensures accurate identification.
- *Chief complaint:* Note the patient's chief complaint or reason for seeking emergency care.
- *Allergies and medications:* Document any known allergies and the patient's current medications. This information is vital to prevent allergic reactions and drug interactions.
- *Vital signs:* Record vital signs, such as heart rate, blood pressure, respiratory rate, temperature, and oxygen saturation, at regular intervals.
- *Interventions:* Document all interventions performed, including procedures, medications administered, and the rationale behind these actions.
- *Response to treatment:* Describe how the patient responds to interventions and any changes in their condition. This information guides ongoing care decisions.
- *Patient statements:* If the patient is conscious and able to communicate, document any statements or information provided by the patient. This may include allergies, medical history, or details about the onset of symptoms.
- *Witness information:* If there are witnesses or bystanders who provided assistance, document their names and contact information in case further information is needed.
- *Legal and ethical considerations:* Be mindful of legal and ethical considerations, such as patient confidentiality and informed consent. Ensure that your documentation complies with relevant laws and regulations.
- *Handoff communication:* When transferring care to another healthcare provider or facility, provide a clear and concise handoff report that summarizes the patient's condition and ongoing treatment plans.
- *Review and corrections:* After the emergency, review your documentation for accuracy and completeness. Correct any errors or omissions promptly.

In an emergency, the focus should always be on providing immediate care to the patient. However, maintaining good documentation practices throughout the process is essential for continuity of care, legal protection, and quality improvement efforts. Documenting critical information during or shortly after the emergency ensures that vital details are not lost.

Healthcare providers should prioritize documenting the most critical information while ensuring that the documentation process does not impede the delivery of lifesaving interventions.

MEDICAL DOCUMENTATION OF TELEPHONIC CONSULTATION AND TELE-CONSULTATION

Medical documentation of telephonic consultations and teleconsultations is essential for maintaining accurate and comprehensive patient records, ensuring continuity of care, and complying with legal and ethical requirements.

Remember that teleconsultations should be conducted with the same level of professionalism, confidentiality, and

documentation diligence as in-person consultations. Thorough and accurate documentation is essential not only for patient care but also for legal and regulatory compliance when providing healthcare services through telemedicine.

BEST PRACTICES IN MEDICAL DOCUMENTATION FOR LEGAL DEFENSE

To overcome these challenges and maximize the role of medical documentation as a defense for doctors, healthcare institutions, and professionals can adopt best practices.

- *Training and education:* Providing training to healthcare professionals on proper documentation practices is essential.
- *Standardized templates:* Implementing standardized templates for documentation can ensure consistency and completeness.
- *EHR integration:* Ensuring that EHR systems are integrated seamlessly and securely can enhance the efficiency of documentation.
- *Quality assurance:* Conducting regular audits of medical records for legibility, completeness, and accuracy can help maintain high standards.
- *Legal review:* Involving legal experts to review and guide documentation practices can be beneficial.

SUMMARY

Medical record keeping is not a mere administrative task; it is a cornerstone of modern healthcare and a linchpin in medicolegal matters. As illustrated by case laws from various jurisdictions, comprehensive and accurate medical records provide critical evidence of patient care, adherence to standards, informed consent, and effective communication. In a healthcare landscape where legal claims are on the rise, healthcare institutions and professionals must recognize the paramount importance of meticulous documentation to safeguard their professional integrity and interests.

By embracing best practices and recognizing the legal significance of documentation, healthcare providers can ensure the highest standards of patient care while protecting themselves from unwarranted legal disputes. As technology continues to evolve, the digital transformation of medical documentation presents opportunities to enhance efficiency, security, and accessibility, further solidifying its role in both patient care and the legal arena.

LEARNING KEY POINTS

- *Legal safeguard:* Medical documentation serves as a crucial legal defense, providing a documented account of patient care that can protect doctors in case of legal disputes or malpractice claims.
- *Evidence of due diligence:* Comprehensive records demonstrate a doctor's commitment to thorough and diligent patient care, showcasing adherence to professional standards and guidelines.
- *Communication clarification:* Clear and accurate documentation enhances communication among healthcare professionals, reducing the risk of misunderstandings and ensuring a collaborative approach to patient treatment.
- *Risk mitigation:* Well-documented medical records serve as a proactive measure in risk management, minimizing the potential for errors, oversights, or misinterpretations in patient care.
- *Informed decision-making:* Thorough documentation provides a basis for informed

decision-making, offering a comprehensive overview of a patient's medical history, treatments, and responses, aiding both current and future healthcare providers.

TAKE-HOME MESSAGE

"Good documentation means a good defense, poor documentation means poor defense, and no documentation means no defense".

MUST AVOID THINGS

Reusing text from previous notes without updating or verifying its accuracy can lead to misinformation.

DO NOT DO

Do not fail to document any known allergies.

WARNINGS

Medical records should be preserved according to legal requirements and institutional policies to prevent loss or tampering.

MUST DO THINGS

Medical documentation is must even during emergency.

ONLY ONE FACT TO REMEMBER

Medical documentation is only defense available for doctors in case of medical negligence.

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Medical Negligence in Pediatrics

Medical Negligence in Pediatrics presents an ongoing set of legal challenges that continually evolve and impact pediatric specialists in their practice. These legal issues continue to catch pediatricians off guard regularly. The use of seemingly harmless terms such as "iatrogenic", "known cases of", "known complications", and "known causes of death or disability" can be linked to allegations of medical malpractice, among other accusations.

Pediatric medical care, vaccinations, and related services, fall under the jurisdiction of the Consumer Protection Act-2019 and the National Medical Commission (NMC) 2019. The NMC introduced a new code of conduct on 02-08-2023, which kept in abeyance from 23-08-2023, resulting in the continuation of existing code of ethics 2002. The Chimera of s. 106(1) of Bharatiya Nyaya Sanhita 2023 (BNS 2023) is apparently harsher than IPC 304A, a way away from complete decriminalization as promised on December 20th, 2023 by Union Home Minister.

For the first time, pediatricians start recognizing the importance of effective communication regarding material risks, proper documentation, counseling, forewarning, risk disclosures, guarantees, and warranties. All of these elements contribute to creating informed consent/contracts for medical services. This book underscores the importance of knowing why, when, how, where, and what to document on outpatient (OPD), inpatient (IPD) transfer, and discharge papers, as this documentation serves as rebuttable evidence through the proper preservation of medical records.

This book is designed in a reader-friendly format that covers practical and relevant topics comprehensively. Each chapter includes sections like "Aim", "Objective", "Summary", "Key learning points", and "one liner crisp Medicolegal punching wisdoms" to facilitate the retention of crucial legal concepts relevant to medical practice. It will prove beneficial to all pediatricians, addressing preventive measures for legal issues in medical practice and providing guidance for those facing litigation. Undoubtedly, this book will be a valuable resource for pediatricians, the judicial system, and practicing lawyers dealing with medical negligence cases. "Medical Negligence in Pediatrics," underscores the urgent need to address the multifaceted challenges faced by physicians in today's healthcare environment. As physicians increasingly confront legal repercussions for errors or adverse outcomes, they are burdened with not only financial liabilities but also personal threats, acts of violence, professional sanctions, and even imprisonment. These consequences often arise from a lack of awareness and adherence to legal principles, casting a shadow over the noble mission of healthcare.

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Foreword
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JAYPEE

CHAPTER 17

Medicolegal Issues in Pediatric Critical Care

Dnyanesh DK, Suma Dnyanesh

Keywords: Emergency treatment, Indian Penal Code, Medical negligence, Medicolegal case (MLC), Pediatric intensive care units

Aim: Pediatric critical care must adhere to a complex body of laws, regulations, and ethical standards. This includes complying with requirements for informed consent, surrogate decision-making, end-of-life care, medical record documentation, and reporting of adverse events.

Objective: Medicolegal issues are prevalent in pediatric critical care, as healthcare providers navigate complex ethical dilemmas, manage critically ill children, and interact with families facing difficult decisions. These issues stem from the delicate balance between providing life-saving treatment, respecting patient autonomy, and adhering to ethical and legal principles.

INTRODUCTION

No greater opportunity, no greater responsibility, and no greater obligation can fall to any other human being than to become a medical professional.[1] In the care of suffering, the medical professionals need scientific knowledge, technical skill, moral understanding of profession, and awareness about the relevant laws of the land. Primarily doctors, especially physicians, dealing with emergency and critical care have several ethical and legal obligations in performance of their duties. The doctors who are involved in critical care should know their legal obligations to avoid allegations and safeguard themselves against medical negligence. This chapter discusses important medicolegal issues in emergency room and critical care.

MEDICOLEGAL ISSUES RELATED TO REFUSAL OF EMERGENCY PATIENT CARE

Case scenario: A 4-year-old child with history of continuous convulsions was brought to appropriate nursing home. But the doctor refused to treat the child because child was requiring mechanical ventilatory care but the patient was poor. So without giving any treatment sent the child to a government hospital. The child died on the way to the hospital. Now can the doctor of the private nursing home be booked under medical negligence?

Explanation: First of all, we should know what medical emergency is. Medical emergency means sudden, unforeseen condition of the patient and/or where the question of life and death is there.[2]

In the case of "Paramanand Katara versus Union of India", the honorable Supreme Court has observed that: "There can be no second opinion that preservation of human life is of paramount importance. That is so on

account of the fact that once life is lost, status quo ante cannot be restored as resurrection is beyond the capacity of man. Every doctor whether at a government hospital or otherwise has professional obligation to extend his services with due expertise for protecting life. No law or state action can interfere to avoid or delay the discharge of the paramount obligation cast upon members of the medical profession".[3]

So in conclusion, "Doctors cannot refuse emergency treatment to a patient irrespective of his caste, creed, ability to pay, etc. because saving human life is of paramount importance. It is a legal obligation on each doctor".[4]

In the above case scenario, doctor knows that it was a medical emergency. He could have intubated the child and given oxygen, anticonvulsants, and IV fluids. Since patient was not affordable, then he could have transported the child to a government hospital where ventilator facility is available. This is the legal and professional obligation.

A doctor is free to choose his patients, say according to their capacity to pay his fees or according to his level of competence. But this liberty does not extend to medical emergency cases, accidents, and injury cases.

Hospitals, nursing homes, clinics of doctors who declare that they provide emergency services are legally bound to attend all medical emergency cases, accidents, and injury cases. They cannot refuse the treatment to such cases.

MEDICOLEGAL ISSUES RELATED TO EMERGENCY MLC CASES

Case scenario: Mr "X" met with an accident. He had head injury and fracture of right femur. He was bleeding heavily and was taken to nearby hospital. But the doctor did not attend the case saying that it is a medicolegal case (MLC) and advised the attendants to take him to a government hospital. But on the way, the patient died. Can the doctor be held responsible for this?

Explanation: In the case of "Paramanand Katara versus Union of India", the honorable Supreme Court has observed that: "There are no provisions in the Indian penal code, criminal procedure code, motor vehicle act, etc., which prevent the doctor from attending the seriously injured persons before the arrival of police. The treatment of the patient would not wait for the arrival of police or for completing the hospital formalities".[5] Hence for the doctors, the cases should not matter from the treatment point of view whether MLC or non-MLC. First priority is to save the life of the patient, next comes the legal formalities. In MLC cases, the only thing the doctor has to do is informing police after stabilizing the patient or after the death of the patient.

Remember, no doctor should refuse to attend the emergency cases, accidents, and injury cases. Try to stabilize the patient as per your skills and competence and refer to higher centers in case if it is required, with suitable mode of transport.

In the above case, the doctor is found to be negligent.

Can government hospitals refuse emergency cases saying "no vacant bed" or specialist doctor is not available?

Case scenario: Mr "M" fell from a height and sustained serious head injury and brain hemorrhage. He was taken to a primary healthcare center (PHC). But since necessary facilities for the treatment were not available, he was referred to a district government hospital. There his admission was refused

saying *"no vacant bed"*. Then he was taken to a state government hospital. There also, he did not get admission saying *"no vacant bed"* and no neurosurgeon is available. Then he was treated in a private hospital with heavy expenditure. Can the state government be held responsible for this?

Explanation: Honorable Supreme Court, in the case of "Paschim Bengal Khet Mazdoor Samity and others versus West Bengal", has stated that Article 21 of Indian constitution imposes an obligation on the state to safeguard the right to life of every person. Preservation of human life is thus of paramount importance. The government hospitals run by state and doctors employed there are duty bound to provide medical assistance for preserving human life. Failure to provide timely medical treatment to a person in need results in violation of his right to life guaranteed under Article 21.[6] So, it is the responsibility of state government to upgrade the hospitals at district and sub-divisional level, so that serious cases can be treated there and government should make sure the all government hospitals are well equipped and adequate staff and specialist are there. Otherwise, government has to pay the compensation.

MEDICOLEGAL ISSUES RELATED TO CONSENT IN CRITICAL CARE

Case scenario:

1. An 18-year-old boy was brought to emergency room with history of severe abdominal pain with distension. Patient was in altered sensorium and was diagnosed to have ruptured appendix with peritonitis and requiring immediate laparotomy surgery to save the patient. The patient attender after leaving him to the hospital was not available for giving consent. Can surgeon take the patient for laparotomy without consent?
2. A 16-year-old boy was admitted in pediatric intensive care unit (PICU) for the treatment of severe broncho-pneumonia. He was in PICU for 10 days. But there was no much improvement in his condition, was on verge of requiring mechanical ventilation. Since the parents were very poor, they were asking for leaving against medical advice (LAMA). But the boy was requesting to continue the treatment including ventilation. Can 16-year-old boy give consent for major treatment?

Explanation: When a patient >12 years approaches a physician, the consent for physical examination is implied. Sections 88 and 90 of IPC 1860 say that any person who is conscious, mentally sound, and >12 years old can give consent for physical examination, diagnosis, and treatment.[7] But when the patient is requiring hospitalization and invasive procedures, written informed consent of the patient is a must. In case if the patient is <18 years, mentally not sound, or is in unconscious state, written informed consent from the parents or relatives or guardian is a must. Otherwise, it amounts to an offence. The doctrine of written informed consent is premised on the notion that "every human being of adult years and sound mind has a right to determine what shall be done with his body".[8]

The informed written consent should include the following things:

- Diagnosis
- Nature and purpose of proposed procedure or treatment

- Risks, side effects, and consequences of proposed procedure
- Reasonably available alternatives and their risks
- Expected outcome without the procedure.

Informed consent should be in the language that patient/relatives can understand and it should not contain any medical or technical language which an ordinary person cannot understand. It should be signed by doctor, patient/guardian, and witnesses. Remember blanket consent is not considered as consent legally.

There are some legally recognized exceptions to the doctrine of informed consent in the context of emergency care.

- Medical emergency where patient cannot give consent, nobody is there with patient and delay in treatment can lead to death of the patient, then the life-saving procedures can be done even without the consent (Section 92 IPC)[9]
- *Section 92 IPC*: The well-being of the patient is of paramount and medical rather than legal considerations come first. No delay in medical intervention is expectable for want of consent.

In the case of Barnett versus Bacharach, the court held that in a medical emergency, in which patient lies unconscious on the operating table, the surgeon may lawfully carry out the duties of a physician in the best interest of the patient, even if these duties entail the performance of a procedure that was not originally contemplated. In Barnett versus Bacharach, a patient who complained of abdominal pains was diagnosed with ectopic pregnancy. The patient was consented only for the removal of ectopic pregnancy. On incision, the surgeon found that the patient did not have ectopic pregnancy but had acute appendicitis. The surgeon in the best interest of the patient, appendectomy was performed. The patient recovered well, but refused to pay the hospital bills, saying she had not given consent for the removal of appendix. The trial court found that the surgeon acted properly because of the seriousness of the patient's condition.[10]

It is essential to note that for the unconscious patient exception to apply, the relevant emergency situation must require immediate medical attention with insufficient time to fully inform the patient or seek consent from another authorized person.

In the case of Tabor versus Scobee, the court found that, a violation of informed consent had occurred. During the surgery of appendectomy, the surgeon found infected fallopian tubes and decided to remove the tubes at that point in the best interest of patient. The court held that removal of tubes in this case did not fall within the exception to informed consent in an emergency situation.[11]

Note that, conscious refusal of the patient in case of emergency will not come under the exceptions to doctrine of informed consent. Physician must respect the refusal of treatment by the patient even during medical emergency.

Physicians are generally not held liable for treating a minor without a parental consent, when an emergency exists. As immediate injury or death could result from the delay, associated with attempting to obtain parental consent. In case of Jackovach versus Yocom, court held that when a minor patient's condition is life-threatening and requiring immediate medical attention, does not require parental informed consent. But in the case of Rogers versus Sells, court held that when a minor patient's condition is life

threatening, yet not requiring immediate medical attention, the emergency exception does not apply. Therefore, parental concern or consent from another legally authorized individual must be obtained.[12]

Younts versus St Francis Hospital is an example of the mature minor exception. In this case, court held that if the minor patient has the ability to understand and comprehend the nature of the proposed treatment as well as associates the risks and potential results in view of surrounding circumstances then law allows the minor to give informed consent.

Emergency blood transfusion in a minor patient: If a minor is in need of life-saving blood transfusion and parents of the minor are refusing because of religious beliefs, the majority of courts may order to transfuse the blood to preserve the life of the child.

MEDICOLEGAL ISSUES RELATED TO NEGLIGENCE OF JUNIOR DOCTORS/POSTGRADUATE STUDENTS

Case scenario: A 10-year-old boy was admitted with severe shock, no IV access for fluids. Since the consultant was out of station, he asked the postgraduate to put central line in the neck. The postgraduate put the line and went for food. After 3 hours when he came back, the child was found to be gasping. Immediately it went into cardiac arrest and died. Whether the consultant is vicariously liable?

Explanation: Improper delegation of responsibility would itself qualify as negligence. Doctors have a duty to satisfy themselves that the persons to whom the work is delegated are qualified and have the appropriate experience to discharge the delegate task.

In "Spring Meadow hospital versus Harjot Ahluwalia", it was held that a consultant could be vicariously liable where he delegates the responsibility to his juniors with knowledge that the junior was incapable of performing his duties properly. Similarly, hospital is liable for the negligence of its staff.[13] In the case of "Cassidy versus Ministry of health", it is stated that the hospital authorities are responsible for the negligence of their staff including nurses, physicians, surgeons, anesthetists, etc. It does not matter whether they are permanent or temporary, full time or part time. Similar observations were made in the case of KK Radha versus GU Shekhar.[14]

In the above case, the consultant is vicariously liable.

MEDICOLEGAL ISSUES RELATED TO TRANSPORT OF EMERGENCY PATIENTS

Most of the doctors will be worried about the transport of accident and critically ill patients to higher centers because of fear of legal process. But in the strict sense, the law requires the victims to be transported even by the nonmedical public and if not, it amounts to negligence. The transport could be accomplished with medicos as even with paramedical people.[15] Even if the patient dies during the transport, the law just requires the matter to be informed to the police personnel, if the death is under suspicious circumstances or if it is an MLC. Naturally as a responsible law abiding citizen, we have to oblige the summons from the court as and when necessary.

MEDICOLEGAL ISSUES RELATED TO TELEPHONIC ADVICE GIVEN BY A CONSULTANT TO A RESIDENT DOCTOR IN ICU, CAUSING HARM TO THE PATIENT: CAN IT AMOUNT TO NEGLIGENCE?

Explanation: It is better to avoid giving medical advices on phone. In case you know about the patient, you may give some instructions and advice on phone to the resident doctors. But ask the resident doctor to write down the instructions and to read it out what he has written, to confirm. Otherwise, any harm caused by telephonic advice amounts to negligence.[5] Best way is to follow Telemedicine Practice Guidelines issued by National Medical Commission.

MEDICOLEGAL ISSUES RELATED TO ISSUING DEATH CERTIFICATE IN "BROUGHT DEAD" CASES

Explanation: This is not an uncommon scenario in emergency rooms. Patient will be brought dead and the attenders will be forcing the doctor to give death certificate. Routinely death certificate is issued by Registered Medical Practitioner who has attended the last stages of life of the patient. So if the patient is brought dead to the hospital or dies unattended, the death certificate cannot be issued. In this situation, it is given after postmortem.[16]

Case of unnatural (MLC) deaths, on table anesthetic and operative deaths, should always be reported to the police for conducting inquest and postmortem. No consent is required for medicolegal PM as it is done as per the requirements of law.

MEDICOLEGAL ISSUES RELATED TO LEAVING AGAINST MEDICAL ADVICE

Explanation: All patients/patient attenders have right to refuse further medical care. They can leave against medical advice to the extent that it does not contravene any law. They have a right to obtain details of investigations and treatment undergone. But the doctor, before sending the patient LAMA, should talk to the patient/patient attenders, regarding the patient conditions and treatment given. Document the discussion in the case sheet properly and take the consent signature of patient/patient attenders.[5]

MEDICOLEGAL ISSUES RELATED TO POST DEATH DIAGNOSTIC PROCEDURE

Case scenario: A 6-month-old child was admitted in PICU with a history of jaundice and hepatosplenomegaly noticed since 1 month of age. Probable diagnosis was storage disorder. The child died after 2 days of admission. Post death, the doctor did liver biopsy without the consent of parents. Is the doctor right?

Explanation: No—Even after death of the patient, if you want to do any procedure, you have to take the consent of parents/relatives. You have to explain them how does this postdeath diagnostic test helps them. The senior doctors often encourage their junior colleagues to "practice procedure" in dead or on dying patients like endotracheal intubation. But before doing such procedures, the sensitivities of the family should be taken into consideration.[4]

MEDICOLEGAL ISSUES RELATED TO "NOT PAYING HOSPITAL BILLS": CAN THE HOSPITAL KEEP THE DEAD BODY TILL THE CLEARANCE OF PAYMENT?

Explanation: Keeping a dead body is not an offence under any sections of IPC. But in some states like Karnataka under KPME Act 2017, it is an offence. So the doctor should not do this. Hand over the body immediately to the relatives and file a civil suit in the court against the relatives who had admitted the patient after signing informed written consent.[15]

MEDICOLEGAL ISSUES RELATED TO A MOB ATTACKING THE HOSPITAL AND DAMAGING THE PROPERTY

Explanation: This is a very common scenario nowadays. If there is any death of a close relative/friend, due to a serious medical problem, some people come in group and attack the doctors, equipment of the hospital and cause heavy damage. To prevent such violence against doctors and hospitals, some of the states like Karnataka, Maharashtra, Tamil Nadu, etc. are having laws, Karnataka Prohibition of Violence against Medicare Personnel and Damage to Medicare Service Institutions Act 2009. According to this Act, any violence against doctor and hospital is a nonbailable cognizable offence and punishment includes imprisonment of up to 3 years with fine. The fine is estimated based on damage, usually double charge of damaged things.[17] This law has really helped the doctors and the states where still this law has not been implemented, it is the responsibility of doctors of that state to put pressure on that government to bring this law. Whenever there is mob attack on the hospital first thing you should do is inform the police and all hospitals should have CCTVs at different places of hospitals so that you will be saving evidences.

MEDICOLEGAL ISSUES RELATED TO WITHHOLDING OR WITHDRAWAL OF LIFE SUPPORT

Case scenario: A 17-year-old patient was admitted in ICU for the treatment of pneumonia and meningitis. Patient was on ventilation for 14 days but there was no improvement. Later his relatives wanted to take him home and requested the doctor to withdraw the life support measures as it was not helping the patient. Legally can the doctor withdraw the life support or does it amount to euthanasia?

Explanation: Legally there is no difference between withholding or withdrawal of life support. Both have same intention to stop futile interventions. Withholding treatment means not starting treatment, withdrawal of treatment means stopping the treatment.

In a recent SC case, Aruna Ramachandran Shanbag versus Union of India, SC has legalized withdrawal of life support measures in patients with persistent vegetative state.[18] It is acceptable for terminally ill cases where further intervention, treatment is futile. But before withdrawing the life support measures, you should discuss with patient relatives, document the whole discussion in the case sheet and take signature on written informed consent form. Rarely in some cases for withdrawal of the life support measures, we need to take permission from high court. Remember that doctor alone cannot take unilateral decision in withdrawal of the life support measures. It amounts to "helping to commit suicide" (IPC sections 306 and 309).

Doctors are expected to give life, not to take it away. Law does not give this power to doctor to act independently. Only after patient relatives consent, you should withdraw the life support. In spite of this, some patient relatives approach the court claiming negligence. Then the IPC sections 76, 81, and 88 protect the doctors (nothing is offence which is done in good faith without criminal intention).

Withdrawal of the life support measures or withholding of the life support measures in terminally ill patients does not amount to euthanasia. Because in euthanasia, the intention is to cause death by some means but in withdrawing or withholding of the life support allows the disease to take its natural course, it may result in death later.

Still, there are no clear guidelines regarding withdrawing or withholding of the life support in terminally ill patients. Hence, it is better to adopt ISCCM (Indian Society of Critical Care Medicine) guidelines.

When you find that further treatment is going to be futile explain the prognosis to patient relatives, if the relatives are ready for the withdrawal of therapy, then record the discussion in the case sheet, take consent signature of the relatives and follow the ISCCM guidelines, i.e.:

- DNR (do not resuscitate)
- De-escalation of therapy
- Do not start any new medication.

With these measures, patient will die in few hours and you can declare the death. Here, the legal problems of withdrawing or withholding of the life support will not arise.

Do not Attempt Resuscitation ICMR Consensus Guidelines

These guidelines help treating physician on their decision concerning do not attempt resuscitation (DNAR). These guidelines preserve dignity in death and avoid prolonged suffering through nonbeneficial CPR (cardiopulmonary resuscitation). Treating physician should initiate discussion with the parent of critically sick child or patient/surrogate and explain in detail about the patients disease, prognosis and benefits, harms of CPR in case patient develops cardiac or respiratory arrest. All discussion must be noted in patient's case records and DNAR form. Good communication and documentation are very crucial. DNAR is distinct from withdrawal or withholding of other life supporting treatment. It guides to continue other curative and supportive care.

Preventive Measures Against Complaint of Medical Negligence in Critical Care

Lord Denning: The doctor's professional reputation is as dear to him as his body, perhaps more so and an action for negligence could wound his reputation as severely as a dagger could injure his body.

A doctor should always keep in mind, the following "3D" principles during practice to avoid a complaint of medical negligence.

- *Duty:* The doctor should do his duty sincerely. Always attend to an emergency and to your patient regularly. Manage the case properly as per the treatment given in standard textbooks. Law is just expecting reasonable care and skills as per your qualification. There may be errors in the diagnosis but it should not be gross. It is the duty of a medical practitioner to keep his professional knowledge up to date as medical science changes day by day.
- *Dialog:* Communication with patient/patient's family is an important part of medical management. Most of the

legal problems arise when there is a communication gap between patient and the doctor. Communication with the patient should be regular and in the language the patient understands. Always communicate the diagnosis, details of treatment, surgery required, likely complications, prognosis, and probable expenditure.

- *Documentation:* A good medical record is the physician's best defense. The case sheet is the important medical record for the treatment of patient in the emergency department as well as in the court of law. The case sheet should have name, father's name, address, and hospital number on each page. All entries should be dated and timed. The physician's notes should not be at variance with that of the nurse. All major events during the patient's stay should be recorded with date and time. A complete well-documented record that convincingly gives the diagnosis and records actions which are appropriate for the diagnosis and the clinical conditions of the patients suggests to the lawyer not to proceed any further. No attempts should be made to change in the record at a later stage.

SUMMARY

To effectively address medicolegal issues in pediatric critical care, healthcare providers should cultivate a comprehensive understanding of ethical and legal principles, establish clear guidelines for navigating ethical dilemmas, promote open communication and collaboration among healthcare professionals and families, seek guidance from legal counsel and ethics experts when faced with complex situations, and continuously evaluate and refine practices to align with evolving ethical and legal standards.

LEARNING KEY POINTS

- *Comprehensive understanding:* Healthcare providers in pediatric critical care must acquire a thorough comprehension of both ethical and legal principles relevant to their field.
- *Guidelines establishment:* It is essential to establish clear and well-defined guidelines for effectively navigating ethical dilemmas that may arise in the course of pediatric critical care.
- *Open communication:* Promoting open and transparent communication among healthcare professionals and involving families in the decision-making process is crucial for ethical and legal practice.
- *Collaboration:* Collaborative decision-making involving multidisciplinary healthcare teams can lead to better ethical outcomes and legal compliance in pediatric critical care.

TAKE-HOME MESSAGES

To always prioritize the best interests and well-being of the child when making medical decisions, and ensure thorough documentation of these decisions.

MUST AVOID THINGS

Do not appoint unqualified and unregistered subordinates.

DO NOT DO

Do not hesitate to take second opinion from qualified specialist when available.

WARNINGS

Restrict to your specialization.

MESSAGES WHICH THE READER MUST BE AWARE

Written informed consent from parents/guardian is a must before each invasive procedure.

MUST DO THINGS

Medical documentation. It is only available defense for doctors in medical negligence.

MEDICAL NEGLIGENCE PEARLS

Always follow standard treatment protocols from standard textbooks and standard guidelines.

ONLY ONE FACT TO REMEMBER

Do not refuse medical emergency cases, accidents, and injury cases.

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Medical Negligence in Pediatrics

Medical Negligence in Pediatrics presents an ongoing set of legal challenges that continually evolve and impact pediatric specialists in their practice. These legal issues continue to catch pediatricians off guard regularly. The use of seemingly harmless terms such as "iatrogenic", "known cases of", "known complications", and "known causes of death or disability" can be linked to allegations of medical malpractice, among other accusations.

Pediatric medical care, vaccinations, and related services, fall under the jurisdiction of the Consumer Protection Act-2019 and the National Medical Commission (NMC) 2019. The NMC introduced a new code of conduct on 02-08-2023, which kept in abeyance from 23-08-2023, resulting in the continuation of existing code of ethics 2002. The Chimera of s. 106(1) of Bharatiya Nyaya Sanhita 2023 (BNS 2023) is apparently harsher than IPC 304A, a way away from complete decriminalization as promised on December 20th, 2023 by Union Home Minister.

For the first time, pediatricians start recognizing the importance of effective communication regarding material risks, proper documentation, counseling, forewarning, risk disclosures, guarantees, and warranties. All of these elements contribute to creating informed consent/contracts for medical services. This book underscores the importance of knowing why, when, how, where, and what to document on outpatient (OPD), inpatient (IPD) transfer, and discharge papers, as this documentation serves as rebuttable evidence through the proper preservation of medical records.

This book is designed in a reader-friendly format that covers practical and relevant topics comprehensively. Each chapter includes sections like "Aim", "Objective", "Summary", "Key learning points", and "one liner crisp Medicolegal punching wisdoms" to facilitate the retention of crucial legal concepts relevant to medical practice. It will prove beneficial to all pediatricians, addressing preventive measures for legal issues in medical practice and providing guidance for those facing litigation. Undoubtedly, this book will be a valuable resource for pediatricians, the judicial system, and practicing lawyers dealing with medical negligence cases. "Medical Negligence in Pediatrics," underscores the urgent need to address the multifaceted challenges faced by physicians in today's healthcare environment. As physicians increasingly confront legal repercussions for errors or adverse outcomes, they are burdened with not only financial liabilities but also personal threats, acts of violence, professional sanctions, and even imprisonment. These consequences often arise from a lack of awareness and adherence to legal principles, casting a shadow over the noble mission of healthcare.

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EPA Navigator

"Charting the Course for Future Pathologists"

A beginner's workbook designed for pathology faculty looking to implement an

Entrustable Professional Activities

-based curriculum as per the National Medical Commission's Guidelines for MD in Pathology

Edited By : Dr. Reshma G. Kini

with introduction by Prof. Olle ten Cate

EPA Navigator

"Charting the Course for Future Pathologists"

Editor: Dr. Reshma G. Kini

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Dedicated to my beloved teachers

Dr. Nagesh K.R, Dr. Shailaja S & Dr. Smitha Bhat

&

Faculty at

Medical Education Unit, Father Muller Medical College, Mangaluru

GSMC FAIMER Regional Institute, Mumbai

NMC Nodal Centre for Faculty Development (ACME), JNMC Belagavi

Preface

Welcome to an enlightening journey through the landscape of Entrustable Professional Activities (EPAs), a transformative approach in medical education that is beginning to take root in our region. This workbook is designed as your guide through the intricate process of understanding, writing, and validating EPAs in pathology, tailored to foster comprehension of an EPA-based curriculum aligned with the curriculum mandated by the National Medical Commission (NMC) for MD in Pathology. It serves as an introductory resource, intended to demystify the fundamentals of EPAs for beginners, setting the stage for deeper exploration and application.

The dawn of Competency-Based Medical Education (CBME) heralds a paradigm shift toward outcomes that truly reflect the capabilities of a medical professional in real-world scenarios. At the heart of this evolution lies the concept of EPAs - units of professional practice, entrusted to learners once they have demonstrated sufficient competence. This workbook you hold is a steppingstone towards equipping you with the knowledge and skills necessary to navigate this shift.

This meticulously curated content begins with an introduction by Prof. Olle ten Cate, laying the groundwork for understanding EPAs within the framework of CBME. Subsequent sections delve into the key principles of EPA-based curriculum, writing a complete description an EPA, assessment strategies, and the crucial process of validation, using the EQual rubric. A carefully selected sample EPA is provided to aid your study and practice, ensuring a comprehensive learning experience.

This is the revised version of the original EPA Navigator which was released at the workshop “EPA -In Focus” conducted by the Department of Pathology, Father Muller Medical College on 30th March 2024. This version has undergone

substantial revision in the chapter on assessment to align with what learning I intend the reader of this book to have. Excepting the introductory chapter by Professor Olle, the content, ideas, and exercises presented within this workbook are the product of my learnings garnered over two years of study of this concept. As the principal author and editor of this workbook, I have curated and significantly contributed to the development of its content, ensuring that it serves as a comprehensive guide to the development of EPAs in postgraduate pathology.

I would like to acknowledge with gratitude the core team of "EPA -In focus" Dr. Nisha J Marla, Dr. Prashanth R, Dr. Chandni Bhandary, Dr. Sueallen D'Souza, Dr. Christina Goveas, Dr. Umashankar T who have been responsible for developing the EPAs a sample of which is provided in the book and for their contributions to the chapters. It has been the collective efforts of this team which has made this book a reality.

My eternal gratitude to Prof. Olle ten Cate for being with us throughout this journey.

Welcome aboard!

Dr. Reshma G. Kini

Editor: EPA Navigator

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Workshop Schedule of "EPA-In focus"

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8:45- 9:00 am	Pre-test	
9:00-9:20 am	Get-to-Know-You Gathering (Icebreaking)	Dr. Sueallen D'Souza
9.20- 10:10 am	Navigating the Intersection: EPAs in Competency-Based Medical Postgraduate Programs	Dr. Reshma G. Kini Dr. Umashankar T
10:45- 11:00 am	Tea break	
11:00-11:45 am	The Anatomy of an EPA Description: Understanding Every Part	Dr. Vijay Shankar S Dr. Christina Goveas
11:45 - 12:30 pm	EQual Rubric	Dr. Reshma G. Kini
12:30-1:15 pm	EPA Assessment Demystified: From Tradition to Entrustment	Dr. Sunita Patil Dr. Prashanth R
1:15 -1:45 pm	Lunch	
2:00- 3:00 pm	Advancing Medical Education: #EPA (Online)	Prof. Olle ten Cate
3:00- 3:45 pm	Workshop on validating EPAs: The EQual Method	Dr. Nisha J. Marla Dr. Chandni Bhandary
3:45- 4:00 pm	Post-test and feedback	
4:00- 4:15 pm	Valedictory and Certificate Distribution	

INTRODUCTION

Prof. Olle ten Cate

Entrustable Professional Activities or "EPAs" as they are commonly known have become a significant component of the design of competency-based education in health professions education. Many postgraduate medical programs and increasingly undergraduate medical programs worldwide are redesigning education and assessment procedures to incorporate EPAs in clinical training. Other health professions have explored the possibilities of using EPAs in their programs, such as nursing, physician assistant, veterinary medicine, midwifery, pharmacy, physical therapy, and dentistry training.

Background

The concept of entrustable professional activities was created to operationalize competency- based medical education (CBME) in postgraduate medical training. CBME frameworks have been highly popular in many countries since the turn of the century but have the risk that they do not translate to day-to-day tasks in patient care. Those tasks may be framed as EPAs. An EPA is a “unit of professional practice that may or should be entrusted to health professions trainees once they have demonstrated to be ready for this responsibility.” This definition, used with slight variations but with the same meaning, stresses that EPAs are the work learners are being trained for.

EPAs may seem just a new method or framework. However, the

potential significance of using EPAs is much wider. EPAs are useful for learners to clarify what tasks trainees must be able to do at the end of training (a postgraduate or undergraduate program, a year, a rotation), at a designated level of supervision. It provides a road map for training in the clinical workplace

EPAs are useful for clinical educators, curriculum developers, and even professional organizations. Defining EPAs is much more than applying a tool. It often is redefining a profession in very practical terms.

EPAs are meant to serve quality and safety in patient care that is provided, both during and after training. By determining a level of supervision that learners require and by certifying learners after adequate assessment, both learners, supervisors, HP colleagues, and patients should have more clarity about what trainees should do and should be allowed to do

EPAs can bridge boundaries across components of an educational continuum (such as undergraduate and postgraduate training) and between clinical disciplines.

EPAs can serve the maintenance of competence. Certification may drop after a period of non-use of skills; and new EPAs may be added after training, to establish a dynamic portfolio of valid EPAs, reflecting clinical competence at any time

State of the art in EPAs

We must realize that, even with its first publication in 2005, the EPA concept is new. Over 90% of all (over 2000) journal publications that used the term 'entrustable professional activity' appeared in 2014 or later (according to Google Scholar). The concept is still being

developed and elaborated, and hardly any program has graduates who were fully trained in curricula with EPAs. Entrustment decision-making and assessment for example has only recently become a topic of scholarly investigation.

Purpose of faculty development for EPA-based curriculum

Identifying suitable EPAs and finding consensus, adapting the objectives and programs of training, implementing new routines for education in the workplace, using entrustment decision-making as an assessment approach, and faculty development to get everyone on board, all require a deep understanding of the EPA concepts and skills and a toolkit of modules and presentations, to be used at local schools and institutions

Is faculty development about EPAs an EPA in itself?

One may wonder whether 'trusting to train others in EPAs' could serve as an EPA. Educational EPAs have become a recent suggestion in the literature, and while teaching activities may not be as critical as health care EPAs, there is a resemblance in the approach. So maybe it would not be bad to think of dissemination of what was learned (i.e. organizing faculty development about EPAs) as an EPA.

Excerpts from

Hennus MP, ten Cate O. EPA Online Course Guide Nov-Dec 2023 [Course handout]. Ins and Outs of Entrustable Professional Activities; [distributed 2023]. Utrecht: UMC Utrecht.

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USED WITH PERMISSION

Prof. Olle ten Cate

Prof. Olle ten Cate attended medical school at the University of Amsterdam in the 1970s and subsequently was employed there as an educational advisor. He completed this Ph.D. dissertation in 1986. In 1999 he was appointed as a professor of medical education at Utrecht University. From 2005 until 2017 he Directed the Center for Research and Development of Education at University Medical Center Utrecht. Since 2018 he is the Scientific Director of Education at UMC Utrecht and is affiliated with University of California San Francisco as adjunct professor since 2012. He was president of the Neth. Association for Medical Education 2006-2012. In 2017 he received the NVMO Han Moll Award and the J.P. Hubbard Award of NBME in the USA, in 2018 the Ian R. Hart Award, and in 2020 a Royal Dutch distinction. Dominant in his current work is competency-based medical education with EPAs.

EXCERPTS FROM THE NATIONAL MEDICAL COMMISSION'S "GUIDELINES FOR COMPETENCY-BASED POSTGRADUATE TRAINING PROGRAMME FOR MD IN PATHOLOGY"

Dr. Reshma G. Kini

Dr. Umashankar T

The National Medical Commission's guidelines for the competency-based postgraduate training program for MD Pathology are aimed to ensure that postgraduate students in pathology are sufficiently trained, professionally competent, and confident in handling, processing, and diagnosis related to histopathology (surgical pathology), cytopathology, and hematology with reasonable working knowledge in blood banking, laboratory medicine, medical statistics, and ancillary techniques with an understanding of general principles and methodology.

How the Document is Framed:

The document begins with broad goals (synonymous with the term **"learning objectives"** in the document). These are then broken down into specific abilities **(Competencies)** that learners need to achieve in the cognitive, psychomotor, and affective domains. The **syllabus** gives a comprehensive list of knowledge and skills to be gained to achieve the competencies defined under the syllabus section.

This framework ensures that the education provided is comprehensive, covering the theoretical knowledge needed to understand pathology, the professional attitudes expected of a pathologist, and the hands-on technical abilities required for the practice of pathology.

The learning objectives, competencies, and skills pertaining to haematology are reproduced exactly as they appear in the NMC document with relevant explanations wherever necessary in order to preserve the meaning that the original authors intended.

Learning objectives

In this framework learning objectives define what the learners need to be able to do at the end of the program. The learning objectives are categorized under four headings:

1. Knowledge of Pathology - 9 objectives
2. Teaching and training - 2 objectives
3. Research- 3 objectives
4. Professionalism, Ethics, and Communication skills

Knowledge of Pathology

- Make a diagnosis based on histopathology (surgical pathology) and cytopathology specimens, blood and bone marrow examination, and various tests of Laboratory Medicine (clinical pathology, clinical biochemistry) as well as Blood Banking (Transfusion Medicine).
- Interpret clinical and laboratory data with reasonable accuracy and prepare a succinct and lucid report
- Compose reports following standard protocols including synoptic reporting
- Interpret and correlate clinical and laboratory data so that clinical manifestations of diseases be explained.

- Advise on the selection of appropriate specimens and tests necessary to arrive at a diagnosis in a problematic case including molecular tests.
- Correlate clinical and laboratory findings with pathology findings at autopsy, identify miscorrelations and the causes of death due to diseases (apart from purely metabolic causes).
- Maintain quality control of all tests by being part of the Internal Quality Control Monitoring program.
- Make and record observations systematically and maintain accurate records of tests and their results for reasonable periods. Identify problems in the laboratory, offer solutions thereof, and maintain a high order of quality control.
- Should be aware of the safe and effective disposal of laboratory waste and ensure the minimization of risk of exposure to infection and accidents to laboratory personnel.

Teaching and training

- Should be able to teach Pathology to undergraduates, postgraduates, nurses, and paramedical staff including laboratory personnel.
- The postgraduate student should be able to teach effectively and assess undergraduate medical and allied health science students so that they become competent healthcare professionals.

Research

- Plan, execute, analyse, and present research work independently or as part of a team.
- The postgraduate student in Pathology should acquire

knowledge and skills to be able to conduct a research project from the planning to the publication stage and become a lifelong learner.

Professionalism, Ethics, and Communication skills

- The postgraduate student should learn and apply principles of professionalism, ethics, and effective communication in the conduct of routine pathology services, research, and routine work.

Competencies

They encompass the detailed descriptions of the knowledge, skills, and attitudes that need to be developed to meet the objectives. Competencies are specific enough to guide learning and assessment. In this document they are categorized into three domains – Cognitive, Psychomotor, and Affective. Though measurable these are broad statements and are listed under various headings.

Example of Competencies:

Cognitive Domain

Hematology

Demonstrate ability to utilize the principles of the practice of haematology for the planning of tests, interpretation, and diagnosis of diseases of the blood and bone marrow.

Conversant with the various equipment used in the hematology laboratory.

Should have knowledge of automation and quality assurance in hematology.

Laboratory medicine

- Demonstrate familiarity with the normal range of values of the chemical content of body fluids, significance of altered values, and interpretation thereof.
- Possess knowledge of the following specialized organ function tests and relative utility and limitations of each and significance of altered values:
- Renal function test
- Liver function test
- Endocrine function test
- Tests for malabsorption
- Principles, advantages and disadvantages, scope, and limitations of automation in the laboratory.
- Learn the principles and methodology of quality control in the laboratory.

Quality Control

- Demonstrate familiarity with various quality control programs running in the department, both internal and external quality.
- Demonstrate familiarity with inert and intra-assay variations, batch variations, and validation of chemicals and instruments.

Laboratory Safety and Good clinical lab practices

- Demonstrate familiarity with good lab practices and safety, record maintenance of capital equipment and consumables, purchase specifications, approximate costs of reagents and equipment, maintenance of store logbooks, etc.

Biomedical Waste Management

- Demonstrate familiarity with disposal methods for each specimen, reagents, instruments, autoclaving techniques, recycling of products, and e-waste.

Affective Domain:

- The student will show integrity, accountability, respect, compassion, and dedicated patient care. The student will demonstrate a commitment to excellence and continuous professional development.
- The student should demonstrate a commitment to ethical principles relating to providing patient care, confidentiality of patient information, and informed consent.
- The student should show sensitivity and responsiveness to patients' culture, age, gender, and disabilities.
- The student should demonstrate a commitment to ethical principles relating to research conduct and research publication.

Psychomotor Domain

Able to collect specimens by routinely performing non-invasive out-patient procedures such as venipuncture, finger-prick, fine needle aspiration of superficial lumps and bone- marrow aspirates, making smears and staining, and providing appropriate guidance to colleagues performing procedures such as a biopsy or an imaging guided biopsy including on-site microscopic assessment of specimen adequacy."

Syllabus

The syllabus provides detailed content (topics, concepts, theories) and practical experiences (laboratory exercises, clinical rotations) that

students will engage with to develop the competencies needed to meet the program's objectives. It is essentially the curriculum, or the educational material and experiences planned to guide students' learning.

Content: The syllabus lists specific topics like general pathology, systemic pathology, hematology, laboratory medicine, etc., which are areas of knowledge students must master.

Practical Experiences: It also specifies the laboratory techniques, diagnostic procedures, and other hands-on experiences students will undergo, such as histological staining, flow cytometry, or conducting research projects.

Syllabus relevant to hematology / laboratory medicine

Psychomotor Domain (Practical Experiences)

III	HEMATOLOGY
1.	Perform venipuncture for peripheral blood collection and decide on appropriate collection tubes, storage, and anticoagulant based on indication
2.	Prepare good quality peripheral blood smears, stain, and report peripheral blood counts and other findings including reticulocyte and platelet counts on the cell counter and manually
3.	Perform bone marrow aspirates and biopsy, prepare good quality smears and imprints
4.	Perform bone marrow aspirate staining including stain for iron
5.	Perform cytochemical characterization of leukemia with special stains on bone marrow aspirates
6.	Perform and interpret coagulation profiles including PT, APTT, and FDP
7.	Perform and interpret the sickling test and osmotic fragility test
8.	Describe accurately the morphologic findings in the peripheral and bone marrow smears, identifying and quantitating the morphologic abnormalities in disease states and arriving at a correct diagnosis in at least common cases referred to the Hematology clinic, given the relevant clinical data

9.	Given the clinical data, interpret the results of i. Red cell indices ii. Plasma hemoglobin iii. Hemosiderin in urine iv. Hemolytic anemia profile including HPLC, Hb electrophoresis, etc. v. Hemoglobin and serum protein electrophoresis vi. Clotting time and other point-of-care tests for bleeding vii. G6PD enzyme estimation viii. Platelet function tests including platelet aggregation and adhesion and PF3 release ix. Russell's viper venom time (RVVT) x. Coagulation Factor assays xi. Serum Fibrinogen xii. Screening for coagulation factor inhibitor, Bethesda Assay, xiii. Fibrin Degradation Products (FDP), D-Dimers xiv. Monitoring of anti-coagulant therapy xv. Thrombophilia profile (Lupus anticoagulant (LAC), Anticardiolipin Antibody (ACA), Activated Protein C Resistance (APCR), Protein C (Pr C), Protein S (Pr S) and Antithrombin III (AT III)) xvi. Serum ferritin, Serum iron, and total iron binding capacity
10.	Interpret flow cytometry findings in the immunophenotyping of leukemia, CD34 enumeration, CD 3/CD 19 enumeration, PNH work up, etc.
11.	Interpret results of cytogenetics and molecular diagnostics in the work up of hematological diseases
IV	LABORATORY MEDICINE
1.	Plan a strategy for laboratory investigation of a given case, given the relevant clinical history and physical findings in a logical sequence, with a rational explanation of each step; be able to correctly interpret the laboratory data of such studies, and discuss their significance with a view to arrive at a diagnosis
2	Perform urine analysis including physical, chemical, and microscopic, examination of the sediment as well as by Dipstick methods.
3	Perform macroscopic and microscopic examination of feces and identify the ova and cysts of common parasites.
4	Perform a complete examination: of physical, chemical, and cell content of Cerebrospinal Fluid (C.S.F), pleural and peritoneal fluid
5	Perform semen analysis and interpret results in the context of clinical and hormone findings

6	Perform quantitative estimation of blood/serum by automated techniques for common biochemical tests
7	Prepare standard solutions and reagents relevant to common biochemical tests including the preparation of normal solution, molar solution, and buffers
8	Interpret and report common laboratory biochemical tests (LFT, KFT, endocrine function tests) with an understanding of clinical implications
9	Operate, maintain, and troubleshoot common equipment used such as photoelectric colorimeter, Spectrophotometer, pH meter, Centrifuge, Electrophoresis apparatus, ELISA Reader, PCR, chemiluminescence, etc.

In concluding this chapter on the National Medical Commission's guidelines for competency-based postgraduate training in pathology, we have delved deeply into the structured and comprehensive approach designed to prepare future pathologists for the challenges and responsibilities of their profession. Through this framework, postgraduate students are expected to develop not only a thorough understanding of pathological conditions but also the practical skills and ethical considerations necessary for their role in patient care and research.

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EPAS IN COMPETENCY-BASED POSTGRADUATE PROGRAMS: NAVIGATING THE INTERSECTION

Dr. Reshma G. Kini

Dr. Sueallen Lorna D'Souza

Background

Overview of Competency-Based Medical Education (CBME)

Competencies in medical education emerged from the realization that traditional models, which were largely time-based and focused on acquiring a broad scope of knowledge and skills, did not necessarily guarantee that all graduates would achieve the desired levels of proficiency in all essential areas of practice.

The CBME model emphasizes outcomes in training, distinguishing itself by focusing on **what learners can do in practice** rather than the time spent in training. The rapid adoption and implementation of CBME across various countries, propelled by frameworks like CanMeds in Canada, the ACGME competencies in the United States, and the Indian Medical Graduate roles in India highlighted a collective endeavor to redefine medical education to meet contemporary healthcare needs.

What are competencies?

Competencies can be simply defined as the abilities required to perform effectively in the workplace. In the context of this book, it pertains to performance of roles and responsibilities of a pathologist.

Competency-based education is a paradigm where education is focused on making the trainees **acquire these abilities**. This approach is built on providing learning opportunities, conducting regular assessments, and offering targeted feedback, all aimed at helping trainees achieve the defined competencies at a desired level of proficiency." The NMC document outlines the specific competencies required for pathology trainees. This standardization ensures that all postgraduate pathology students across the country develop a consistent set of skills and abilities.

Caveat: Competencies alone do not guarantee that the trainee can perform in real-world settings. To assess the abilities(competencies) it is imperative to put them in action (tasks) so that their presence and their level of proficiency can be assessed. This is where the EPAs come in.

The Role of EPAs

The Intersection of Competencies and EPAs

Entrustable Professional Activities (EPAs) are tasks derived from the fundamental responsibilities of a pathologist. Only those tasks that meet specific, established criteria are selected as EPAs. These tasks are then comprehensively described to ensure clarity and relevance within a training context. An EPA based curriculum is a paradigm where trainees are educated and evaluated on their ability to execute these tasks until they can be trusted to carry them out on their own.

It is understood that to perform such a task successfully the pathologist would have acquired the competencies required to perform it. Similarly, for a trainee the ability to perform these tasks independently serves as an indicator of possessing the required competencies. In other words, entrustment of EPAs happens only after the trainee has

demonstrated that he/ she has acquired the competencies required to perform them.

Example:

EPA: Reporting bone marrow aspirate smears

Competencies necessary to perform this task successfully include:

a. Ability to interpret slides and arrive at a diagnosis
b. Ability to correlate clinically
c. Ability to generate a comprehensive report
d. Ability to generate differential diagnosis and perform advisory services for management of the patient

The trainee is entrusted with this EPA reporting of bone marrow aspirates independently *after* she has demonstrated that she has acquired proficiency in the competencies a-d.
Essentially, EPAs make the competencies concrete by connecting them directly to tasks that trainees must be able to perform in their pathology work. When a trainee is entrusted with a set of tasks that cover most of a pathologist's duties, it signifies they are prepared for real-world professional practice.

EPAs hence provide an alternate route to achieving competency-based education by looking at the performance of real -world tasks and embodies outcome- based education.

Identifying and developing EPAs- The steps

1. **Listing Professional Activities** - In the daily practice of pathology, trainees are expected to perform a variety of tasks that mirror the responsibilities of a fully qualified pathologist. Identifying these tasks

involves analyzing the routine activities of a pathologist, for instance, work done over a week.

2. **Verifying Suitability for EPA Development:** Given the resource-intensive nature of EPAs, it is essential to refine the list of potential activities. Each activity should be evaluated to ensure it is not merely a competency or skill, but a discrete task with a clearly defined beginning and end, appropriate in scope, and one that can feasibly be entrusted to a trainee during their training period.

Identifying professional activities includes ascertaining that they are distinct both in process and timing and are observable in process and measurable in outcome. The outcome of the potential EPA should contribute meaningfully to patient care, healthcare processes, or educational goals. Additionally, these activities must be central to the profession, require training in a pathology postgraduate program to be able to perform and align with the competencies outlined by the NMC.

Distinguishing Competencies, and EPAs: A useful approach to distinguish competencies from EPAs is to frame potential EPAs with the phrase, "**Tomorrow I am going to allow you to**…"

Distinguishing Skills and EPAs: The aspects that distinguish the two are compared in the table below:

Aspect	**Skills**	**Entrustable Professional Activities (EPAs)**
Scope	Narrow, focusing on specific, often isolated tasks.	Broad, encompassing a range of tasks and responsibilities that reflect the professional activities of a specific healthcare role.
Integration	Usually involves single competencies or discrete actions.	Requires the integration of multiple competencies (knowledge, skills, and attitudes) across different domains.
Example	Performing	Grossing

	phlebotomy	
Assessment of Competence	Focused on the technical proficiency of the skill performed.	Based on the ability to integrate and apply a range of competencies in a real-world setting; often involves direct observation, feedback, and a judgment of trustworthiness for autonomy.
Educational Focus	Skill development and technique perfection.	Development of professional identity, autonomy, and readiness to handle the complexities of professional practice.

3. **Writing a complete description**: We have chosen to follow the eight-part description as recommended in AMEE guide 140.

Each EPA is description includes its specifications and limitations, potential risks in case of failure of its performance, the most relevant competency domains it is mapped to, the attributes required to achieve entrustment, the information sources that will inform the entrustment decision, and the point of time in the training period at which entrustment of this EPA is considered suitable.

4. **Validating EPAs**:

a. **The first step of validation is to agree upon the list of EPAs**. Elements discussed include the number of EPAs, the title, the breadth of EPA, and whether the list encompasses most of the work performed by a pathologist.

b. **The second step is validating the description of each EPA**. It is recommended that validation be done at the state, regional, or national levels. Several methods have been suggested of which the Delphi technique is considered quite popular. However, we chose to validate our EPAs by expert consensus at the workshop where each component was discussed and later scored using the EQual rubric.

This development process ensures that EPAs are designed to represent the majority of a profession's elements, facilitating the transition from training to real-world practice by emphasizing the acquisition of trust

through demonstrated proficiency in these key activities. However, it's acknowledged that not all-important professional tasks can be directly translated into EPAs.

The Entrustment Process

Beyond Abilities Entrustment is not solely about assessing a trainee's ability or skill; it encompasses a broader evaluation of the trainee's attributes, including integrity, responsibility, reliability, and humility. Entrustment also depends on the supervisors' attributes – their comfort with entrusting with the activity, their concerns for patient safety, the legal implications, and famously the 'gut feeling'

Supervision Scale

The Supervision Scale is integral to the implementation of Entrustable Professional Activities (EPAs) within Competency-Based Medical Education (CBME). This scale facilitates the transition of learners from novice to proficient practitioners by defining and measuring the levels of independence they can safely be allowed when performing specific professional tasks. The scale operates on the principle that the more a supervisor trusts a trainee to perform an EPA, the less supervision the trainee will need.

The Supervision Scale has five levels, each representing a different degree of independence and responsibility the learner can handle. Given here is the generic entrustment/supervision scale.

Level 1 - Observation Only: The learner is not yet ready to perform the activity, even under direct supervision. They are allowed to observe

the task being performed by a competent practitioner.

Level 2 - Direct, Proactive Supervision: The learner can perform the task under direct supervision, with the supervisor physically present and actively guiding the process.

Level 3 - Indirect, Reactive Supervision: The learner may perform the task with the supervisor not physically present but available on demand. This level implies that the learner has achieved a degree of proficiency that allows for some independence, with the supervisor intervening as needed.

Level 4 - Supervision Only as Needed (Unsupervised Practice): The learner can perform the task independently without the need for immediate supervision. However, they are expected to seek advice or help when the situation goes beyond their level of competence. **At this level, the trainee is awarded a STAR- Statement of awarded responsibility.**

Level 5 - Supervision of Others: The learner has mastered the task to the extent that they can supervise and teach others. This level signifies a full entrustment and recognizes the learner not only as competent but also as a potential educator for their peers or more junior learners.

Bryant et al. developed an entrustment scale specifically for anatomic and clinical pathology. They categorized EPAs into three groups: procedure-based tasks, clinical problem-solving tasks, and reporting-based tasks, with five levels of entrustment defined for each category.

Utilizing the Supervision Scale within the context of EPAs provides a robust framework for guiding learners through their educational journey, ensuring that they achieve the competencies necessary for safe and effective practice. This scale underscores the dynamic nature of

learning and the need for ongoing assessment and feedback in the development of professional competence.

Types of entrustment decisions

Ad Hoc Entrustment Decisions

Ad hoc entrustment decisions are made at the moment and are based on the immediate assessment of a learner's ability to perform a specific task or activity safely and effectively and rely on the supervisor's immediate judgment and the specific circumstances at hand. These decisions are often made during routine clinical encounters, where a supervisor decides whether a learner can undertake a particular task under their supervision at that time. Although ad hoc decisions are essential for day-to-day clinical education and provide learners with growth opportunities, they are inherently more variable and subjective.

Structural Entrustment Decisions: Formally moving a PG from one level to the next level of supervision based on predefined criteria.

Summative Entrustment Decisions

Summative entrustment decisions are more formal and reflective, made after a comprehensive evaluation of the learner's accumulated evidence of competence over time. These decisions involve a deliberate and often collaborative process where faculty members review a learner's performance across multiple encounters and assessments to determine their readiness to perform specific Entrustable Professional Activities (EPAs) independently or with minimal supervision.

Summative decisions are typically documented and have significant implications for the learner's progression, signifying a milestone

achievement that the learner has developed the necessary competencies to be entrusted with greater responsibility. Unlike ad hoc decisions, summative entrustment decisions are less about the immediate context and more about the learner's overall preparedness and reliability to carry out professional tasks safely and effectively in their future practice.

Implementing EPA-Based Curriculum

An EPA-based curriculum revolves around formulating EPAs that cover the breadth of a profession's responsibilities, providing trainees with opportunities to develop the necessary competencies for a summative entrustment.

Integrating EPAs into medical curricula requires a deliberate and structured approach to curriculum development, assessment, and feedback. A program of assessment, a program of documentation and evaluation, and establishing criteria for entrustment decisions – all require conducting multiple faculty development programs and a robust plan of implementation

Conclusion

EPAs are pivotal in bridging the gap between competency-based medical education (CBME) and the practical application of skills in clinical settings. By delineating the transition from acquiring broad knowledge and skills to demonstrating specific competencies through actionable tasks, EPAs operationalize competencies, ensuring medical trainees are not just theoretically proficient but also practically capable. Through this integration, medical education can effectively develop competent, autonomous, and responsible healthcare professionals ready for the challenges of modern medical practice.

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THE ANATOMY OF AN EPA DESCRIPTION: UNDERSTANDING EVERY PART

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Entrustable Professional Activity (EPA) is a unit of professional practice that may be entrusted to a learner unsupervised, once he or she has demonstrated the required competence.
Detailed descriptions of EPAs foster a common understanding among students, educators, and regulators about the requirements and expectations for each activity. This clarity aids in curriculum design, learner assessment, and the safe, effective delivery of healthcare, allowing educators to tailor training to individual progress and readiness. This chapter details the description of an EPA as recommended by Taylor *et al.* in AMEE guide 140.

The eight parts of the full EPA description constitute:

1. Title

The title of an Entrustable Professional Activity (EPA), should reflect a clear and focused activity, ideally encapsulating a single, specific professional task. The title should employ continuous verb forms to indicate ongoing activities, emphasizing that the entrustment is continuous and not restricted to a single instance. It is crucial to avoid

ambiguity and ensure the title does not suggest multiple potential interpretations. Lastly, the title should be concise, capturing the essence of the activity in the fewest words possible while still being clear and understandable to all stakeholders involved in medical education and practice

2. Specifications and Limitations

Specifications detail exactly what postgraduate (PG) trainees are entrusted to do under each EPA, akin to inclusion criteria in research. This informs all the stakeholders what different components come under the EPA which is not evident from the title alone. E.g. the EPA – Reporting bone marrow aspirates and biopsies may include staining of the slides and troubleshooting quality issues in the slide as specifications. These are necessary for successful reporting but are not evident in the title.

Limitations, like exclusion criteria, delineate the boundaries of the EPA, clarifying contexts (like atypical presentations/ critically ill patients), diseases (rare diseases), or procedures not covered under this EPA so that it is evident to the stakeholders what the PG is NOT allowed to do independently.

3. Potential Risks in Case of Failure

Highlights the risks associated with failure in executing an EPA. Simply means – what is going to happen if a trainee performing at level 4 supervision fails to do the activity as expected? Writing this down brings the following points into focus

For a trainee- this awareness helps prepare them for real-life scenarios, emphasizing the critical nature of their responsibility's potential

consequences of their actions

For the teacher, it emphasizes the importance of understanding the potential risks involved when entrusting a postgraduate with the EPA, reinforcing the need for careful consideration in the decision to grant autonomy.

4. Competency Domains

EPAs are derived from the competencies that can be placed in various competency domains. Aligning EPAs with relevant competency domains, such as those outlined by ACGME, CanMEDS, and the roles of Indian Medical Graduates, facilitates structured learning and assessment, guiding both educators and learners.

A competency domain is very similar to what is called a role. E.g. what are the different roles a doctor must play to be able to be a competent physician?

In India, the NMC has described roles for undergraduate medical education- Clinician, Communicator, Lifelong Learner, Leader, and member of healthcare team and Professional.

We have at present chosen to map our EPAs to the competency domains of The Accreditation Council for Graduate Medical Education (ACGME)

The ACGME specifies six core competency domains that medical residents and fellows, including pathologists, are expected to develop during their training.

Patient Care (PC): In pathology, patient care involves the accurate and timely diagnosis of diseases through examination of tissues, cells, and body fluids. It also includes performing procedures such as fine needle aspirations and bone marrow biopsies.

Medical Knowledge (MK): Pathologists must possess a

comprehensive understanding of various disease processes, their pathophysiology, laboratory techniques, laboratory workflow, knowledge of quality assurance.

Practice-based Learning and Improvement (PBLI): Continuous self-improvement is crucial in pathology. Pathologists are expected to engage in ongoing education to enhance their skills in diagnostic and procedural techniques, including quality assurance programs that ensure the accuracy and reliability of laboratory results. Learning from previous cases and adapting to new evidence and technologies form the backbone of practice enhancement.

The EPA reporting bone marrow aspirate would be mapped to this domain because it requires continuous practice and updating one's knowledge and skill to be able to do it successfully.

Interpersonal and Communication Skills (IC): Effective communication in pathology involves not only the clear reporting of diagnostic findings but also discussing procedural aspects and results with clinical colleagues. When performing procedures such as FNACs, pathologists must also communicate effectively with patients, explaining the process.

Professionalism (P): High standards of professionalism are essential for pathologists. This includes the ethical conduct of procedural interventions, ensuring informed consent is obtained and that patient dignity is maintained during procedures. Professionalism also entails meticulous adherence to quality standards and regulatory requirements to ensure patient safety and confidentiality.

Systems-Based Practice (SBP): Understanding the healthcare system's workings is vital for pathologists, particularly in terms of how their

services affect and integrate with patient care pathways. This includes coordinating with other healthcare providers to ensure timely and accurate diagnostic and procedural services and improving systems through quality assurance measures that enhance patient outcomes and laboratory efficiency.

Differing systems in which a pathologist may function is in a medical college versus the corporate setup. The demands and the responsibilities may differ significantly, and a pathologist should be able to navigate both to perform effectively.

The ACGME Competency domains and the IMG roles are comparable.

Indian Medical Graduate Role	Description	ACGME Competency Parallel
Clinician	Competence in understanding and managing clinical problems.	'Patient Care' and 'Medical Knowledge'
Leader and Member of the Healthcare Team	Leadership, teamwork, and effective collaboration in healthcare settings.	'Systems-Based Practice' and 'Interpersonal and Communication Skills'
Lifelong Learner	Continuous self-learning and staying updated with medical advances.	'Practice-Based Learning and Improvement'
Professional	Upholding ethical principles, professionalism, and sensitivity to patient needs.	'Professionalism'
Communicator	Effective communication with patients, families, and the healthcare team.	'Interpersonal and Communication Skills'

5. Required Knowledge, Skills, and Attitudes (K/S/A) and experiences

Identifying the K/S/A necessary for each EPA enables trainees to understand what they are expected to learn in each of the domains. It also details the experiences that they need to have to gain an entrustment (e.g. number of times a particular procedure needs to be done) It helps educators in designing targeted learning experiences and assessments. Knowing these in detail can also aid immensely in giving targeted feedback.

6. Information Sources to Assess Progress and Support Summative Entrustment

Multiple sources of information are vital for robust entrustment decisions. This section specifies the types and quantity of assessments that underpin sound entrustment, ensuring decisions are based on a comprehensive evaluation of the learner's abilities.

These would include multiple sources.
For e.g.
The information sources to inform entrustment decisions for the EPA related to bone marrow reporting would include a combination of supervised learning events like daily reporting, personal activities, logbook entries, portfolio entries, performance in case discussions, written tests, and Entrustment-Based Discussions (EBDs). This would also include multisource feedback to assess professionalism and reflective summaries by the student evaluating experiences, highlighting learned skills, and identifying areas for continued growth.

These methods collectively contribute to a holistic assessment of the learner's progress, competencies, and readiness for increased levels of entrustment throughout the training in bone marrow reporting.

7. Levels of Supervision and Entrustment

Defining the expected levels of supervision and entrustment at different stages of training provides a roadmap for progressive autonomy. This structured approach helps trainees understand their developmental trajectory and supports educators in making informed decisions about when trainees are ready to advance to higher levels of responsibility.

8. Time period to Expiration

Acknowledging that competencies may degrade without practice, this component emphasizes the need for ongoing practice and potential recertification. Setting expiration timeframes ensures that trainees and practitioners remain competent over time, maintaining the quality and safety of patient care.

The above 8 sections of a full description of an EPA are not just a theoretical construct, but each of them is a practical and attainable entity that will eventually directly impact the quality of medical education and patient care.

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EPA ASSESSMENT DEMYSTIFIED: FROM TRADITION TO ENTRUSTMENT

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Introduction:

The Indian medical education system historically focused on a time-based model both in undergraduate and postgraduate curricula. Competence was determined based on a single summative examination at the end of the course.

A gap observed between the aspirational goal of producing competent medical practitioners and the structure of the educational programs prompted the adoption of the Competency-Based Medical Education (CBME) model for both undergraduate and postgraduate training, introduced in 2019 and 2022, respectively

- This shift to CBME necessitates the development of new assessment models that align with the principles of assessment in CBME given here:
- **Alignment with Competencies**: The assessments directly link to specific competencies that learners must achieve.
- **Integration across the Curriculum**: The curriculum features continuous assessment to monitor and support learner progress.
- **Authenticity:** The assessments mimic real-life scenarios to ensure

relevance to professional practice.

- **Formative and Summative Assessments:** The model utilizes both ongoing assessments for feedback and final assessments to evaluate competence.
- **Feedback**: Assessors provide timely, constructive feedback focused on behaviors and improvement.
- **Multiple Methods and Sources**: The approach employs a variety of assessment tools and perspectives to gain a comprehensive view of learner competence.
- **Standard Setting and Reliability**: The model establishes clear performance standards and ensures assessments are consistent and fair.
- **Learner-Centeredness**: The assessments engage learners actively in the assessment process, including self-assessment and reflection.
- **Programmatic Assessment**: The program systematically collects data from various assessments over time for high-stakes decisions on learner progression.
- **Continuous Improvement:** The assessment process undergoes regular reviews and refinements based on feedback and data analysis.

Adopting Competency-Based Postgraduate Medical Education (CBPGME) necessitates following the principles that ensure the educational model accurately evaluates the actual performance of postgraduate trainees. This evaluation targets the highest level of Miller's Pyramid, which focuses on demonstrating skills in real-world settings.

To address this, the concept of Entrustable Professional Activities (EPAs) has been introduced. EPAs are tasks or responsibilities to be entrusted to the unsupervised execution of a trainee in the real world. EPAs provide a structured way to assess whether a trainee can carry out core professional activities safely and independently, thus directly measuring their readiness for clinical practice.

Alignment of EPAs with key principles of assessment in CBME

EPAs are task-oriented, directly aligning with specific competencies that learners need to acquire, thereby ensuring that assessments are closely tied to the skills and knowledge required in clinical practice. The authenticity of EPAs, which are designed to mirror real-life clinical responsibilities, supports the principle of authentic assessment, facilitating learners' ability to apply their knowledge in practical, professional settings. Moreover, the use of EPAs encourages a variety of assessment methods and sources, including direct observation and feedback from multiple stakeholders, which enhances the comprehensiveness and reliability of the assessment process. By focusing on the entrustment of professional activities once a learner has demonstrated sufficient competence, EPAs inherently promote a learner-centered approach, encouraging active participation, reflection, and self-assessment. This method of assessment not only supports the transition to a CBME curriculum by aligning with its core principles but also fosters a more holistic, practical, and flexible approach to evaluating the readiness of future medical professionals to meet the demands of their roles.

Assessments in EPA- based curriculum:

The assessment of EPAs can follow different philosophies, including the use of milestones, entrustment scales, or both. Milestones track a trainee's progress through specific objectives, while entrustment scales rely on the assessor's judgment of the trainee's readiness for unsupervised practice. A variety of assessment tools support these approaches, capturing different dimensions of EPA performance.

When reading literature on EPAs it is extremely important which system the authors are referring to in order not to get confused.

A. Assessment through milestones:

Milestones are descriptions of what the PG should have the ability to do as they move from the novice to the expert level within a competency domain/ a competency or a sub-competency.

One example of this framework is the ACGME Milestone Project where the milestones are described for each sub-competency within each competency domain. The progress of learners within each sub-competency is documented from Milestone levels 1 to 5 (not to be confused with levels of supervision). Achievement of milestone is documented and reviewed with periodically.

Some of the medical education bodies have incorporated EPAs within this framework. In this the EPAs are structured around competencies and/or the sub-competencies, with the premise that each EPA encompasses various underlying competencies/ sub-competencies. The notion is that once a postgraduate (PG) resident has attained the requisite proficiency level in each of these sub-competencies (i.e. when the PG reaches the level 4 milestone in each of the sub-competencies)

they would then be considered prepared to execute the EPA autonomously.

B. Assessment through supervision scale

In this school of thought, the focus is on direct observation and the use of entrustment scales to make decisions about a learner's readiness to perform EPAs with minimal supervision. Rather than relying on predefined milestones, which may imply a more rigid and linear progression, entrustment scales are based on the assessor's judgment of the learner's abilities, considering their consistency, reliability, and the context in which the activity is performed.

Entrustment scales often include five levels, from observation only, without any responsibility for the task, to performing the task independently and even being able to teach others. This approach emphasizes the gradual and qualitative increase in trust in a learner's abilities, often integrating multiple sources of evidence over time to inform the entrustment decision. It is a more dynamic and potentially more individualized way of assessing competence.

The Assessment Methods for EPAs

The assessment tools can be divided into seven categories based on entrustment decisions and principles of workplace-based assessment (WPBA)

S. No	Assessment categories	Examples
1	Knowledge tests	Written assessment: SAQs, MCQs

2	Simulation tests	OSCE with standardized patients
3	Short-practice observations	Mini CEX, DOPS, OSATS
4	Long-practice observation	MSF, Mini peer assessment tool, Ward Evaluations
5	Case-based discussion	CBD
6	Product evaluation	Audit assessment, Logbooks, Portfolios, Reports
7.	Entrustment Based Discussions	EBDs

More important than having a list of assessments is to choose the appropriate assessment method that is likely to capture different dimensions of performance of an EPA.

One of the ways to choose the assessments for an EPA could be by focusing on the assessments tools which measure the constructs of each competency domains that the EPA has been mapped to.

Assessment Methods by Competency Domains

1. Patient Care (PC)

1. Direct Observation of Procedural Skills (DOPS): Observing and evaluating residents' performance during specific clinical procedures.
2. Clinical Encounter Cards: Quick feedback tools used immediately after clinical encounters.
3. Workplace-Based Assessments: Evaluating residents' clinical skills and patient interactions in their usual work settings.
4. Simulation-Based Assessments: Using simulated environments to assess clinical skills and decision-making.
5. 360-Degree Feedback: Collecting feedback from multiple sources

including peers, supervisors, and other healthcare staff.

6. Logbooks or Case Logs: Keeping detailed records of cases handled and procedures performed.

2. Medical Knowledge (MK)

1. Regular and Cumulative Written Exams: Testing knowledge through structured exams with various question formats.
2. Oral Examinations: Assessing knowledge and clinical reasoning through verbal questioning.
3. Image-Based Testing: Evaluating diagnostic skills using medical images, slides, or digital simulations.
4. Journal Clubs and Case Presentations: Presenting and discussing current research and clinical cases.
5. Slide tests

3. Practice-Based Learning and Improvement (PBLI)

1. Audit and Feedback: Reviewing and discussing personal or group performance data.
2. Self-Directed Learning Projects: Personal learning initiatives based on self-identified needs.
3. Portfolio Assessment: Documenting and reflecting on learning experiences and professional development.
4. Quality Improvement Projects: Participating in projects aimed at improving healthcare quality and outcomes.
5. Reflective Practice: Writing about personal experiences and learning in clinical practice.
6. Research Projects: Involvement in and evaluation of clinical or laboratory research projects.

4. Interpersonal and Communication Skills (IC)

1. Standardized Patient Encounters: Interacting with trained actors in simulated clinical scenarios.
2. Team-Based Assessments: Observing and evaluating communication within healthcare teams.
3. Patient/Peer/Staff Feedback: Collecting opinions on communication skills from various individuals.
4. Mini-CEX (Clinical Evaluation Exercise): Short, focused assessments of clinical encounters
5. Patient satisfaction survey
6. Portfolio Assessment: Including reflective pieces on communication challenges and successes.

5. Professionalism(P)

1. 360-Degree Feedback: Gathering comprehensive feedback on professional behavior.
2. Self-Assessment and Reflection: Reflecting on and assessing one's own professional conduct.
3. Professionalism Mini-Evaluation Exercise (P-MEX): Specific tool for assessing professional behaviors.
4. Observed Clinical Interactions: Monitoring and evaluating interactions with patients and colleagues.
5. Discussion of Ethical Scenarios: Engaging in hypothetical situations to assess ethical decision-making.

6. Systems-Based Practice (SBP)

1. Participation in Multidisciplinary Meetings/Hospital Committees: Engaging in systemic healthcare discussions and decisions.
2. System Improvement Projects: Involvement in projects to improve healthcare systems.
3. Root Cause Analysis Participation: Analyzing and understanding

the underlying causes of system failures.

4. Healthcare System Navigation Exercises: Demonstrating ability to coordinate complex patient care across systems.
5. Case Management Discussions: Discussing and analyzing the management of patient cases.
6. Cost-Effectiveness Analysis: Understanding and application of cost-effective practices in pathology.

Additional Techniques

1. Peer Assessments: Receiving feedback and evaluations from fellow residents.
2. Online Learning Modules and Quizzes: Using digital platforms for learning and assessment.
3. Performance in Externships or Rotations: Evaluating skills and knowledge gained in specialized training settings.
4. Case Logbooks: Keeping detailed records of clinical cases and procedures for review.
5. Logbooks or Case Logs: Maintaining logs of clinical activities and experiences for assessment.

Making an entrustment decision using assessments

A predefined criteria to say what the PG is expected to perform/ know/ experience at each level to be promoted to the next level on the entrustment scale needs to be established beforehand.

For e.g. We have defined the following criteria to promote a postgraduate from level 1 to level 2 of supervision. The PG should be able to

PC-stain of bone marrow smears with routine and special stains

MK-identify all the normal cellular component in a bone marrow aspirate smear

MK and IC- Describe the components of a bone marrow report and can describe what is to be written under each of the without leaving out any subsection.

P- Should have attended 80% of the daily reporting

The assessment methods would be-

a. DOPS- Direct Observation of Procedural Skills- where we will observe the trainee staining in real time (this task can be delegated to senior PGs or technologist), (PC)

b. Do an Entrustment Based Discussion (EBD) to ask for knowledge regarding things that can go wrong in staining and how to manage them, (PC)

c. Do a slide test or interpretation test to check for correct identification of cells (PC/ MK)

d. Check for completeness of bone marrow report formats of report written independently by the PG (we are not looking for accuracy of the interpretation just checking for the completeness of format) (C/MK)

As the trainee progresses to second year, we define that the trainee should be able to clinically correlate the bone marrow findings and give accurate bone marrow report in 50% of cases and have knowledge of basic hematological disorders to move him from level 2 to level 3. Here we would be focusing on

a. Giving a slide test to check for interpretational proficiency (PC)

b. Will do a case-based discussion to check for clinical correlation (MK/PC)

c. Give a written test to check if reading is happening (MK)

d. Do product evaluation to look for how the PG is framing the reports (we are now looking to check for some accuracy and also for clarity of the report written) (IC)

e. Do a 360-degree evaluation to know about the professionalism and behaviour of the PGs with colleagues, staff both teaching and non-teaching and patients (P)

Summative entrustment decision (awarding Level 4 / STAR) which gives the postgraduate the privilege of performing the EPA independently needs to be robust. This would require multiple assessments by different assessors using multiple tools. Additionally, information on the ARICH criteria also needs to be gathered.

Program of Assessments

It becomes obvious from the above example that each EPA would require several assessments at different points of time possibly by a different teacher as the PG progressed through each EPA. This would require a robust schedule of assessments what we are referring to here as program of assessments. A program or schedule is necessary to ensure the supervisors and trainees remain informed of the frequency as well as the type of assessments that need to be done at each level of supervision.

It's also necessary that the assessments be accompanied by feedback.

Since there may be approximately 25 EPAs within the program the

number of assessments would run in hundreds, and these are to be multiplied by number of PGs in all three years which would make it into thousands of data points per year.

App-based systems have been developed for ease of tracking in a few institutions.

Conclusion:

Though many assessment tools have been described for providing feedback or formative assessment, research on their usage to make summative entrustment decisions is under process. Nevertheless, the several existing assessment tools can support the entrustment decision process

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ENTRUSTMENT BASED DISCUSSION

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Entrustable professional activities (EPAs) are defined as units of professional practice that reflect the responsibilities or tasks that health professionals perform in their daily clinical work. The concept was developed to bridge the gap between competency frameworks and the practical assessment of clinical tasks, acknowledging that many competencies are context-dependent and challenging to assess in isolation.

The central thesis of the document is the significance of entrustment decisions in the workplace, which are crucial for determining when learners are ready to perform clinical tasks with reduced supervision. These decisions are made based on the learners' demonstrated skills, the complexity of the patient's condition, and the acceptable level of risk. Entrustment-based discussions (EBDs) are proposed as a tool to assess learners' readiness for entrustment, focusing on the learner's performance, understanding of the task, anticipation of risks and complications, and adaptability to different situations.

Entrustment-based discussions (EBDs) are carried out as structured conversations between a supervisor and a trainee, focusing on evaluating the trainee's readiness to perform specific Entrustable Professional Activities (EPAs) with less supervision. Here's a detailed

breakdown of how an EBD is conducted:

Introduction to the Case or Activity: The trainee begins by describing what they have done about a specific EPA. This allows the learner to present the case and its challenges, demonstrating their understanding and application of knowledge in a real-world context.

Exhibit Understanding: The supervisor asks the trainee to explain the rationale behind their actions, including pathophysiology and clinical reasoning. This part aims to assess the trainee's depth of understanding and ability to apply theoretical knowledge to practical situations.

Risk and Complications Assessment: The trainee discusses the risks and possible complications involved in the EPA. This section is crucial for evaluating how well the trainee can anticipate potential problems and prepare for them, showcasing their ability to manage risk effectively.

Adaptability to Different Situations: The discussion explores how the trainee would handle the EPA if the patient or situation were different, such as variations in culture, medical history, or unexpected findings. This part tests the trainee's flexibility and adaptability, important traits for dealing with the unpredictable nature of clinical practice.

Feedback and Conclusion: The conversation concludes with the supervisor providing feedback, focusing on the trainee's strengths and areas for improvement. The supervisor assesses the trainee's overall performance, readiness for less supervision, and suitability for specific levels of responsibility. This feedback aims to guide the trainee's future learning and development.

Key differences between case-based and entrustment-based discussions

Aspect	Case-Based Discussions (CBDs)	Entrustment-Based Discussions (EBDs)
Primary Focus	Focuses on assessing the trainee's knowledge, clinical reasoning, and competence based on their handling of a specific patient case.	Focuses on assessing the trainee's readiness to perform specific Entrustable Professional Activities (EPAs) with less supervision, including their ability to manage risks and adapt to variations.
Context of Evaluation	Usually based on the information from a patient's (electronic) health record and revolves around a previously handled case.	While it may involve discussing a specific case, the emphasis is on evaluating the trainee's overall readiness for entrustment with EPAs in various situations.
Assessment of Risks	While risk management can be discussed, it is not the central focus. The discussion may touch on potential complications but not necessarily the trainee's ability to anticipate or manage these risks.	Explicitly includes risk assessment as a crucial component. Trainees must demonstrate their preparedness to manage risks and complications, showing a proactive understanding of potential challenges.
Flexibility and Adaptability	Primarily retrospective and specific to a case; does not explicitly focus on the trainee's adaptability to different clinical situations or variations in patient conditions.	Proactively assesses how the trainee would adapt to different scenarios, including variations in patient conditions, cultural differences, and unexpected findings, emphasizing adaptability.
Outcome and Feedback	Outcome focuses on identifying knowledge gaps and areas for improvement based on the specific case discussed. Feedback is oriented towards enhancing specific competencies.	The outcome focuses on making entrustment decisions regarding the trainee's readiness for specific EPAs. Feedback includes readiness for unsupervised practice and areas for growth in managing responsibilities.

Sample EBD

EPA title: Reporting routine bone marrow aspirate

What have you done?

Describe the process you followed for the bone marrow reporting.

Can you walk us through your involvement in the bone marrow examination and reporting?

Exhibit understanding:

What indications led you to recommend or perform a bone marrow examination?

How do you interpret the findings of a bone marrow report in the context of this patient's condition?

Explain the pathophysiological implications of the abnormal bone marrow findings.

Potential Risks

What are the potential consequences of misinterpreting bone marrow analysis results?

How would you handle a situation where the bone marrow sample is inadequate or compromised?

Can you identify any risks associated with delayed reporting or miscommunication of bone marrow findings?

Discuss the implications of overlooking critical findings in the bone marrow report. How would you mitigate such risks?

In the event of an inconclusive bone marrow report, how would you communicate the need for further testing to the clinical team?

Describe the steps you would take if you noticed a significant discrepancy between the current and previous bone marrow reports of the same patient.

How would you manage a situation where there is an unexpected delay in the processing or reporting of bone marrow results?

What if Questions

How would your reporting change if the bone marrow sample showed unexpected findings that could suggest a rare condition?

What if the patient had a complex medical history with multiple comorbidities; how would this affect your analysis and reporting?

If you encountered contradictory findings between the clinical picture and the bone marrow report, what would be your next steps?

Imagine you are reporting bone marrow findings in an urgent case; how would you ensure the accuracy and timeliness of your report?

If you were presented with a bone marrow report during a time-sensitive case, how would you prioritize the information to be communicated?

How would you approach to reporting change if the bone marrow analysis indicated the possibility of a hereditary blood disorder?

What if you had to explain the bone marrow report to a colleague from another specialty; how would you ensure they understand the implications for patient care?

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EPA VALIDATION: A STRUCTURED APPROACH

Dr. Reshma G. Kini

The EQual rubric is a structured tool designed to align Entrustable Professional Activities (EPAs) with the established standards of competency-based medical education (CBME). Its purpose is to ensure that EPAs are clearly and precisely defined, enabling accurate assessment, reliable prediction of future performance, and appropriate determination of the level of entrustment that can be granted to a learner for a specific task.

Why validate an EPA?

Ensuring Alignment with Educational Goals: Validation ensures that the EPA accurately reflects the competencies and skills it is intended to measure. This alignment is essential for the EPA to be a meaningful and effective tool in assessing a learner's readiness for a particular aspect of professional practice.

Reliability and Consistency: Validating an EPA helps to establish its reliability. This means that different assessors would likely arrive at similar conclusions when evaluating the same learner on the same EPA, thereby ensuring consistency in assessment.

Determining Appropriateness and Feasibility: Through validation, educators can determine if an EPA is appropriate for the stage of training and feasible to be implemented within the educational setting. This includes considering the resources required for assessment and

whether the EPA can be integrated effectively into the existing curriculum.

Identifying Areas for Improvement: The process of validation can help identify aspects of an EPA that may need refinement, such as clarifying language, adjusting complexity, or ensuring it addresses all relevant competencies.

Supporting Program Evaluation and Accreditation: Validation of EPAs contributes to the overall quality assurance of a training program. It demonstrates a commitment to high educational standards, which is essential for program evaluation and accreditation processes.

The EQual rubric consists of 14 items (criteria) against which an EPA is validated. Each of the 14 items can be rated from 1 to 5 on a Likert scale except for item no. 1.

The fourteen criteria fundamentally evaluate the EPA in three constructs

Task Validation: Confirming the inherent value and appropriateness of the task which has been chosen to be framed into an EPA e.g. performance restricted to qualified personnel, performance of the task requiring training within the profession.

Outcome Validation: Evaluating the outcome of the EPA concerning

- Whether there is a tangible output/ outcome at all.
- If yes, then is it relevant for the trainee (as per NMC norms)
- If yes, then is it valuable enough to be made into an EPA (Is it entrustable?)

Framing Validation: Assessing the clarity and accuracy of the task's

description.

Criteria for EPA Validation as per EQual Rubric

1. This EPA has a clearly defined beginning and end

This talks about the discreteness of the task

EPAs must exhibit a clearly defined beginning and conclusion, ensuring tasks are distinguishable in both process and time. This specificity allows for assessing the task's completion and helps in the attribution of outcomes to the task. It also helps in determining performance and the outcome/output effectively.

Example:

1. Ensuring the quality of reports vs
2. Troubleshooting quality issues in the haematology laboratory

Task 2 when assigned is more discrete than Task 1

2. This EPA is specific and focused

Precision in defining EPAs is crucial, especially when entrusting patient care to trainees. Detailed documentation is necessary to delineate what tasks trainees are authorized to undertake, minimizing potential risks to patient safety.

Examples:

1.Managing the laboratory during night shifts versus
2.Addressing technical and critical issues during after-hours duty

Point 2 is telling the trainee as well as the supervisor what is expected

from the trainee in more specific terms. The description of the technical and critical issues mentioned in the title will need further elaboration in the full description of the EPA document under the heading of "specifications and limitations."

3. This EPA is independently executable to achieve a defined clinical outcome

The EPAs should be structured such that the task is self-contained, and its completion does not rely on the completion of other tasks.

A very good example would be

1.Writing reports of bone marrow aspirate vs

2.Reporting (Signing out) bone marrow aspirates

To execute the first, the trainee would first need to analyze the slide, correlate the findings with the clinical parameters, and seek additional information if necessary. These activities DO NOT come under the purview of the task – writing reports of bone marrow aspirate (which is narrow and small enough to qualify as a skill rather than an EPA)

Whereas the title - Reporting bone marrow aspirate will encompass all the activities mentioned.

NOTE: The clinical outcome here is the report that is generated. But the way the EPA is framed to make it into a stand-alone activity is of prime importance.

4. This EPA is observable in the process

The ability to observe tasks in action is vital for direct assessment. It is closely linked to the fact that we are also going to want to attribute the output of the task to the performance of the task then the task itself

should be observable in the process.

Example:

1. Should have a thorough knowledge of automation in hematology
2. Reporting of bone marrow aspirates

In the first example, the knowledge is not observable though having this knowledge is more than mandatory. Also, note it's not a task but an ability. This criterion is an additional safeguard against writing competencies as EPAs. Competencies (loosely translated as abilities are not observable unless put in action)

In the second example the EPA is not entirely observable in the process i.e. actual interpretation of the slide is not observable in the process. The outcome of how it was interpreted and how it was reported can be measured.

In such a case, though you may rate task 2 higher it would still not get full 5 points on the scale. You can rate it lower on this parameter.

Note: Tasks that are observable in the process tend to be procedural.

5. This EPA is measurable in the outcome

An effective EPA should yield a tangible, quantifiable output. This characteristic is key to evaluating outcomes

Example:

1. Communicating effectively vs
2. Heading a clinical pathological meeting

Broad Competency vs. Specific Task:

Communicating effectively is a broad competency that, while

important, presents challenges in measurement due to its subjective nature. To accurately assess this skill, one must define "effective communication" within each specific context it occurs, which can vary widely.

In contrast, “leading a clinical pathological meeting” represents a specific task where the ability to communicate effectively is tested in a real-world scenario. This approach allows for the observation and evaluation of communication skills in action, providing a more concrete and measurable outcome than a general competency description.

By focusing on specific tasks or scenarios, such as managing a clinical pathological meeting, educators can create clearer, more objective criteria for assessing communication skills, thereby enhancing the overall reliability and validity of the evaluation process.

6. Performing this, EPA leads to recognized output or outcome of labour

This reiterates the above point of EPA needing to have a tangible output. The outcome of labour can be in relation to patient care/healthcare process / related to the educational objectives of the trainee. This criterion means that the performance of the EPA MUST CONTRIBUTE an output to one of the three areas. EPAs that contribute directly to patient care would be something like reporting a frozen section. The report (output) is directly linked to patient care. An example of output related to the healthcare process could be an EPA titled troubleshooting technical issues in haematology. Though this does not directly contribute to patient care nevertheless is still an extremely important task within the healthcare process. An EPA that is neither directly related to patient care or healthcare process but is

very important from the point of view of their training would be an EPA titled -performing research or teaching.

It is important to note that there might be many other things that the PGs are required to do during their pathology postgraduate training which is not directly linked to any of these three areas. In case of doubt always fall back upon the NMC document. EPAs are extremely resource and time-intensive - if the NMC document says that they need to do it, then they need to do it and if it does not exist within the NMC document, then the best is to avoid it making it into an EPA at least initially.

7. This EPA addresses professional work that is suitable for entrustment

This criterion might initially be the most challenging to interpret due to potential ambiguities arising from its English translation. At first glance, the term "entrustable" might suggest a focus on whether a task can be assigned to a trainee. However, the true intent of this criterion is to evaluate the task's inherent value and significance within professional practice. Specifically, it assesses whether the task is crucial enough to warrant its designation as an EPA, reflecting tasks that patients would reasonably expect a pathologist to perform. Therefore, "entrustable" in this context relates to the task's relevance and the quality of its output, highlighting the necessity of ensuring that each EPA is not just doable by a trainee but is fundamentally important and adds meaningful value to the field.

This criterion assesses the significance of the task's output, ensuring it adds value to professional practice.

8. This EPA is clearly distinguished from other EPAs in the

framework

For an Entrustable Professional Activity (EPA) to effectively contribute to a learner's development, it must be distinct and differentiated from other EPAs within the educational framework. To provide a robust and comprehensive educational journey, each EPA must stand out distinctly from others, eliminating any overlap. This distinctiveness ensures that each EPA targets a specific skill set or body of knowledge, contributing uniquely to the learner's overall competency development. This ensures that each EPA provides a unique learning opportunity, covering the full spectrum of skills and responsibilities relevant to the profession.

Example: Imagine two EPAs, one focused on "Performing bone marrow aspiration analysis" and another on "Diagnosing hematological malignancies." At first glance, these may appear to cover different aspects of pathology. However, significant overlap exists since diagnosing hematological malignancies often relies on the analysis of bone marrow aspirates. If not clearly distinguished, these EPAs could lead to redundant training on bone marrow analysis techniques, overshadowing broader diagnostic strategies for hematological malignancies that also involve peripheral blood smears, genetic testing, and clinical correlation.

9. This EPA describes work that is essential and important to the profession

EPAs should represent tasks fundamental to the profession, reflecting their critical nature in medical practice.

10. The performance of this EPA in clinical practice is restricted to qualified pathologists

EPAs are designed to reflect tasks that necessitate the specialized expertise unique to the field of pathology. These tasks underscore the importance of the specialized training and qualifications pathologists receive, distinguishing their capabilities from those of other healthcare professionals. This criterion emphasizes that only those with specific pathology training can competently perform the task described by the EPA, underscoring the role's specialized nature.

11. This EPA Requires the Application of Pathology-Specific Knowledge, Skills, and Attitudes (KSAs)

This point reinforces that the execution of the task demands the application of comprehensive KSAs acquired through pathology training. The integration of these two criteria implies that the task is not just exclusive to pathology by nature of its specialization but also because it requires the application of pathology-specific training that cannot be substituted by a general medical background.

Consideration of Overlap with Other Specialties:

While some tasks may appear to overlap with those performed by other healthcare specialties, such as bone marrow procedures or interpreting coagulation tests, it's essential to evaluate how central these tasks are to the identity and core competencies of a pathologist. In instances of overlap, tasks may be rated lower in terms of exclusivity but should still be considered for inclusion if they are deemed essential to the practice of pathology. This approach assists in prioritizing EPAs, particularly when aiming to maintain a manageable number for a postgraduate program, recommended to be around 30. The primary criterion for inclusion should be whether a task is foundational to the role and responsibilities of a pathologist, regardless of whether similar tasks are performed by professionals in other disciplines.

12. This EPA involves the application and integration of multiple domains of competence

EPAs should encourage the application of a wide range of competencies, enhancing the educational value of the task.

13. The EPA title describes a task, not qualities or competencies of a learner

Example: Knowledge of automation in hematology is a critical competency but should be framed as a task-oriented EPA for clarity and measurability.

When in doubt see if the EPA will complete the sentence –

"Tomorrow, I will allow you to_____"

14. EPA describes the task and avoids adjective (or adverbs) that refers to the proficiency

When describing tasks, avoid qualifiers that imply a level of proficiency, focusing instead on the task itself.

Example: "Interpret bone marrow aspirate slides competently."

Conclusion:

A structured approach to EPA validation ensures that EPAs are designed effectively, focusing on clear, observable, and measurable tasks that align with educational and professional standards. This clarity enhances the training experience, ensuring that EPAs serve their intended purpose in competency-based medical education.

Dr. Reshma G. Kini

SAMPLE EPA

EPA No. 7	Sample EPA
Title	Reporting routine bone marrow aspirate smears
Specifications	The EPA encompasses 1. Interpretation of routine bone marrow aspirates and biopsies 2. Writing comprehensive bone marrow aspiration and biopsy reports as per the standard format including prognostic indicators, and any recommendations for further testing or treatment. 3. Ensuring quality of slides, reports, and troubleshooting issues.
Limitations	Complex cases requiring multiple opinions from consultants Complex cases are those with: **a. Unexpected neoplasms** b. **Atypical Findings**: Cases that exhibit rare or atypical cell morphology not commonly encountered in routine aspirates. c. **Diagnostic Uncertainty**: Cases where the cellular pattern does not point to a well-defined pathological process or disease state. d. **Multiple Pathologies**: Cases that suggest the presence of more than one underlying hematologic condition or disease.
Potential Risk in case of failure	Patient harm (Wrong treatment, delayed treatment), Legal issues
Most relevant Competency Domains	1. **Patient Care**: accurate interpretation for patient management. 2. **Medical Knowledge**: Applying in-depth knowledge of pathology and hematology to accurately diagnose and provide differential diagnoses from bone marrow samples. 3. **Interpersonal and Communication Skills:** Effectively communicating findings, verbally and through reports clearly and concisely. 4. **Practice-Based Learning and Improvement**: Continuously enhancing personal skills and knowledge in bone marrow analysis and adapting to new findings and techniques.

Most relevant Sub competencies as per ACGME's Pathology milestones	**Patient Care 1**: Reporting **Patient care 3**: Clinical Consultation including On-Call Interactions **Patient Care 4**: Interpretation and Diagnosis **Medical Knowledge 1**: Diagnostic Knowledge **Medical Knowledge 2**: Clinical Reasoning **Practice-Based Learning and Improvement 1**: Evidence-Based Practice and Scholarship **Practice-Based Learning and Improvement 2**: Reflective Practice and Commitment to Personal Growth **Interpersonal and Communication Skills 2**: Interprofessional and Team Communication
Knowledge skills and attitudes required	1. **Knowledge of hematological disorders** 2. **Interpretation skills** Accurate and efficient interpretation of bone marrow aspirates and biopsies. 3. **Clinical reasoning skills**: a. Ability to correlate laboratory findings with clinical data. b. Engaging in analytical thinking to interpret results within the broader context of patient care. c. Competence in providing differential diagnoses based on laboratory findings and clinical reasoning to provide advisory services 4. **Communication and Reporting skills**: a. Writing clear, concise, and accurate reports on findings. b. Interacting with healthcare teams regarding laboratory results and offering advisory services to healthcare professionals 5. **Professionalism and Ethics**: a. Adherence to the highest standards of professionalism and ethics in all aspects of laboratory work and reporting. 6. **Technical Proficiency**: a. Mastery in staining techniques for bone marrow aspirates. 7. **Ensuring quality** of slides, reports and troubleshooting issues
Information sources to assess progress and support summative	Product evaluation= stained slides, formulated reports, Evaluations of Case based discussions (day to day and formal) Evaluations of Entrustment based discussions

entrustment decision*	Results of Slide and written test results Journal Club evaluation Multisource feedback Portfolio review Logbook review
Level 4 to reach at which time in the three years	At 2.5 years into residency
Time period to expiration	2 years

***PLAN OF IMPLEMENTATION AND INFORMATION SOURCES TO SUPPORT ENTRUSTMENT DECISIONS**

Levels	Week/s	Supervised learning events	Personal Activities	Logbook entries	Portfolio Entries
Level 1	1 to 4	Attendance in daily reporting	Observation of 10 bone marrow procedures Observation of 10 cases of bone marrow staining Interpret slides for identifying normal marrow elements.	Entry of 10 cases of observatio n of staining and BM procedure	Pictures of stained slides
	4 to 8	Daily reporting to attend to and identify cellular elements Assist in staining at least 15 cases	Read the bone marrow chapter on normal cellular elements and attempt to identify normal elements	Entry of cases stained	At least 5 case histories are to be written in brief with the final diagnosis.
Level 1 to Level 2					
	End of week 8	80% attendance in regular reporting Able to identify normal marrow elements 50% of the time		Logbook entry of BM procedure s seen, staining observed, and log of cases seen	Pictures of stained slides Reflective summary, evaluating their experiences highlighting learned skills, and identifying areas for continued growth
Level 2	8 to 18	Attendance in regular reporting Case	Attempt clinical correlation in the majority of cases Read chapters on	Log of all the cases observed, the case	At least 5 case histories of the bone marrow were done.

		discussions on a day-to-day basis Do 2 EBDs (to be done by seniors) (this is to be followed by a feedback session Present 1 hematology case discussion (this is to be followed by a feedback session Written test on hematology disorders	hematological diseases (syllabus to decide)	presented	Description of the case presented with the learnings Description of cases presented in the EBDs with the feedback given Same with the heat case discussion
Level 2 to Level 3					
	End of week 22	80% attendance in reporting Score above average in the hematology case discussion Score average on all parameters in interpretation rubrics Score 75% marks in the written test given	NA	Log of all activities	Descriptions of cases with clinical history, clinical diagnosis, pathological findings, and clinical correlation in 5 cases. Scores of both rubrics are to be maintained Attach EBD evaluation with feedback given Submission of a reflective summary, evaluating their experiences highlighting learned skills, and identifying areas for continued

					growth
Level 3	22 to 23	Attendance in regular reporting Present 1 Hemat case discussion Present 1 Medicine ID Present 1 JC Do at least 5 EBDs during regular reporting	Write comprehensive reports and provide DDs and advisory services	Log of activities Log of EBD cases, Hemat case discussion, JC, and ID with scores obtained	A summary of cases for which BM performed ID done Case discussions done Write a letter to the editor after a critical analysis
Level 3 to Level 4					
	End of week 32	Pass in written test Score good or above in the interpretation rubric Score acceptable in EBD rubric for 4/5 cases		Logbook review	Portfolio review Submit a reflective summary, evaluating their experiences highlighting learned skills, and identifying areas for continued growth
Level 4	Week 33 onwards	To maintain all activities as described in Level 3 To lead one Medicine Interdepartmental case discussion emphasizing collaborative diagnosis and treatment planning	To clear the model exam in haematology	Log at least 10 cases	Attach complete BM reports

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With warm regards and deep appreciation

Reshma

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Dr. Reshma G. Kini

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Art and Science of Breastfeeding & Beyond

Durgappa H
Anita Nyamagoudar
CR Banapurmath

Foreword
VD Patil

JAYPEE

CHAPTER 13

Feeding in Preterm and Low Birthweight Infants

Manisha Bhandankar, Tanmaya Metgud, Durgappa H

"Being at the breast reminds the baby of being in the womb:
there's that familiar heartbeat and soothing voice, as well as the warmth and comfort.
And that makes the transition to the outside world a little easier"

—Jack Newman

INTRODUCTION

Low birthweight (LBW) is an important risk factor contributing to high perinatal mortality and long-term morbidity. LBW neonates weigh <2,500 g and are further classified as "Very low birth weight" if they weigh <1,500 g and "Extremely low birth weight" if weighing <1,000 g at birth. These neonates can be result of preterm birth (before completion of 37 weeks), small for gestational age (SGA) (<10th centile weight for gestation), or both.

Approximately 8 million newborns are delivered LBW in India every year contributing to 18% of total birth as per the National Family Health Survey (NFHS)-5 (Year 2019–21).[1] Previous chapters have already highlighted the importance, benefits, and role of exclusive breastfeeding in newborns. In this chapter, we will discuss current recommendations with regards to feeding LBW and Preterm infants. Most of these recommendations are from the Clinical Practice Guidelines prepared by the National Neonatology Forum in January, 2020.[2]

Feeding in small vulnerable newborn (SVN) babies; preterm, LBW, and small for gestation is particularly challenging due to various maternal factors, immature sucking and swallowing reflexes and coordination, baby's specific nutritional needs, and limitations with feed tolerance after birth. This chapter discusses the type of milk, timing, quantity, and modes of feeding among LBW infants.[3]

GENERAL PRINCIPLES

All neonates are required to be breastfed immediately after birth to induce lactation, prevent hypoglycemia, and establish mother-and-baby bonding. LBW and preterm neonates are in particular at higher risk of hypoglycemia due to lower glycogen stores in the liver at birth. Clinically stable LBW neonates weighing >1,800 g and more than 34 weeks' gestation can be managed at mother's side immediately after birth provided, they are adequately fed and closely monitored for hypoglycemia and temperature maintenance. Among LBW neonates born by

cesarean section, it is likely that mother may not be able to start breastfeeding immediately after birth. In such cases, available suitable options should be adopted.

Which milk?

Currently, we have three options for neonates:

1. MOM: Mother's Own Milk
2. DHM: Donor Human Milk
3. Formula Milk.

Low birthweight infants including those with very low birth weight (VLBW) and Extremely low birth weight (ELBW) should be fed MOM. Those, who cannot be fed mother's own milk should be fed DHM (recommendation relevant for settings where safe and affordable milk-banking facilities are available or can be set up). Those who cannot be fed MOM or DHM should be fed standard infant formula (recommendation relevant for resource-limited settings).

Very low birthweight infants who cannot be fed mother's own milk or donor human milk should be given preterm infant formula if they fail to gain weight, despite adequate feeding with standard infant formula. VLBW infants who fail to gain weight despite adequate breast milk feeding should be given human milk fortifiers, (HMF) preferably those that are human milk based.

How?

- *Direct breastfeeding:* Stable LBW infants weighing more than 1,800 g and >34 weeks' gestation
- *Katori/spoon feeding:* Stable LBW and VLBW infants with gestational age >32 weeks. These infants may require additional intravenous (IV) fluids due limited feed tolerance in the first few days.
- *Gavage feeding:* Clinically unstable LBW, VLBW, and ELBW infants may require additional IV fluids to meet their nutritional and fluid demands. Bolus intermittent feeds are well tolerated in most groups.

Gavage feeding in neonates: Neonates who are not mature enough to suck and swallow feed but are stable enough to receive feeds can be started on tube feeds [Neonates with gestational age <32 weeks, late preterm and term neonates with respiratory distress, on continuous positive airway pressure (CPAP) or ventilator].

Placement of tube: Feeding tube can be inserted through oral cavity [orogastric (OG) tube] or nasal cavity [nasogastric (NG) tube]. There is no clear benefit of one route over the other. However, most neonatal units prefer OG tube placement.

Benefits and disadvantages of one route over the other can be discussed.

Length of NG tube/OG tube insertion is calculated by distance from bridge of nose-to-ear lobe and then from ear lobe to midway between xiphisternum and umbilicus (NEMU method).[4] Correct tube placement is checked by first aspirating and then pushing in 2 mL air and listening by stethoscope. Tube feeding lowers sucking function and its motor development in premature neonates.

There is no significant difference in somatic growth and incidence of necrotizing enterocolitis (NEC) between NG or OG feeding methods has been reported.[5] Third hourly feeding is recommended for infants weighing >1,250 g. Those weighing <1,250 g, second hourly feeding can be given. Once the infant is stable and ready for oral feeding, paladai feeding should be introduced.

- *Paladai feeding:* Studies have reported that premature neonates with over 30 weeks of gestational age and physiological stability can be safely fed by paladai and show better weight gain than cup feeding. Cup/paladai feeding is useful for the neonates

who have good swallowing ability but do not have appropriate sucking. It is also recommended for those neonates who cannot be breastfed to stimulate sucking by providing the neonate experience of oral feeding.

- *Oral swabbing:* Application of MOM to oral mucosa in extremely preterm neonates who are on ventilator or clinically unstable have been reported recently.[6] The outcome of the study like prevention of ventilator-associated pneumonia needs elaboration.

When?

Clinically stable LBW infants weighing >1,800 g and are able to breastfeed should be put to the breast as soon as possible after birth. These infants and mothers may need extra support to initiate and establish breastfeeding. The infant may be fed every 2–3 hourly or based on hunger cues.

Stable VLBW infants can be started on gavage or katori feeding within 24 hours of birth. Currently, there is lot of variation in practices for feeding preterm infants between 28 and 32 weeks and birthweight between 1,000 g and 1,500 g. Nangia *et al.* have reported that early total enteral feeding in stable VLBW infants is feasible and safe with decrease in NEC, sepsis, and hospital stay.[7]

MINIMAL ENTERAL NUTRITION

Sucking and swallowing coordination is known to be established between 32 and 34 weeks in preterm infants. Practice of feeding small volume of enteral feeds in order to stimulate development of immature gut is known as "Trophic Feeding" or "Minimal Enteral Nutrition". Initiation of trophic feeding at 10–20 mL/kg in hemodynamically stable VLBW newborns on first day (Expressed breast milk MOM or DHM) and progression of feeds at 20–30 mL/kg every day to reach target feeding at 150–180 mL/kg has not shown increase in the incidence of NEC, feed intolerance (FI), and combined outcome of NEC and FI. Slow feed advancement with delayed establishment of full enteral nutrition has been reported to be associated with increased chances of invasive infection.[8]

Abnormal antenatal Doppler study is a major factor that influences decision regarding early initiation and slow progression of feeds in VLBW and ELBW infants. Early enteral feeding (as early as 24 hours) can be initiated in Reversal of End Diastolic Flow (REDF) if there are no abdominal symptoms and signs.[9] Among preterm SGA babies with <29 weeks of gestation who have AREDF (Absent/Reversal of End Diastolic Flow), extreme caution has to be exercised while advancing feeds during the first 10 days of life.

NON-NUTRITIVE SUCKING

The National Neonatology Forum India recommends non-nutritive sucking (NNS) should be encouraged in preterm VLBW infants admitted in neonatal unit. This can be done in <32 weeks baby by providing sterile pacifier to suck while tube feeding or encouraging baby to suck on empty breast (after expression of milk) before feeding the baby. The World Health Organization (WHO) has recommended NNS as intervention to reduce the time to transition from gavage to full oral feeding, the time to transition from initiation of oral feeding to full oral feeding, and length of hospital stay.

METHOD OF MILK EXPRESSION

Mothers whose preterm infants are admitted in the neonatal unit should be encouraged to use either breast pumps (manual or electric) or perform manual expression as early as

possible after birth to stimulate lactation. They should be counseled to regularly to express milk even when the infant is not on complete enteral nutrition to ensure higher volume of expressed breast milk as and when required; they may use manual breast pumps to do sequential expression (i.e., expression from one breast followed by that from the other) in the second week after delivery.

Mother should be sitting comfortably after washing hands. She can place a warm clean towel on the breast and gently massage the breasts till milk starts flowing. Then making a C-shaped hold on the areola of the breast, she gently squeezes the breast tissue simultaneously holding a sterile cup in front of the nipple so that milk can drip in it. Skilled professional support should be provided to lactating mothers whose babies are admitted in neonatal units to establish and sustain breastfeeding.

Low-cost interventions such as breast massage, breast warming, and relaxation techniques should be used to enhance breast milk supply in mothers whose preterm infants are admitted in the neonatal unit.

HUMAN MILK SUPPLEMENTS

Multinutrient fortification of breast milk can be initiated in preterm LBW infants with birthweight <1,800 g and receiving enteral feeding of at least 50–80 mL/kg/day.

The VLBW infants should be given vitamin D supplements at a dose ranging from 400 IU to 1,000 IU/day until 6 months of age.

The VLBW infants who are fed MOM or DHM should be given calcium (120–140 mg/kg/day) and phosphorus (60–90 mg/kg/day) supplementation during the first months of life.

The VLBW infants fed MOM or DHM should be given 2–4 mg/kg/day iron supplementation starting at 2 weeks until 6 months of age.

Daily oral vitamin A supplementation and routine zinc supplementation for LBW infants who are fed MOM or DHM is not recommended at the present time, because there is not enough evidence of benefits to support such a recommendation.

How to express breast milk by hand?

- Encourage the mothers to express breast milk by herself.
- Thorough hand washing is important to prevent contamination of expressed milk.
- Let the mother be comfortable in sitting position and hold a wide-mouthed clean container near her breast.
- Massage the breast gently for few minutes. Allow her to lean forward so that gravity is facilitating.
- Put her thumb on her breast above nipple and areola and her first finger on the breast below the nipple and areola, opposite the thumb. She supports the breast with her other fingers. Note that the thumb and index finger are approximately 4 cm from areola across the nipple making a C.
- Press her thumb and first finger slightly inwards toward the chest wall. Use rhythmical backward (Pushing back into chestwall) then forward movements to massage the breast.
- Apply the steady pressure inward toward the chest wall, not toward the nipple.
- Press and release, press and release as shown in the **Figure 1**.
- This should not hurt, if it hurts, the technique is wrong. At first no milk may come, but after pressing few times milk starts to drip out. It may flow in streams if the oxytocin reflex is active.
- Press the areola in the same way from the sides, to make sure that the milk is expressed from all the segments of the breast. She must press on the lactiferous sinuses beneath the areola.

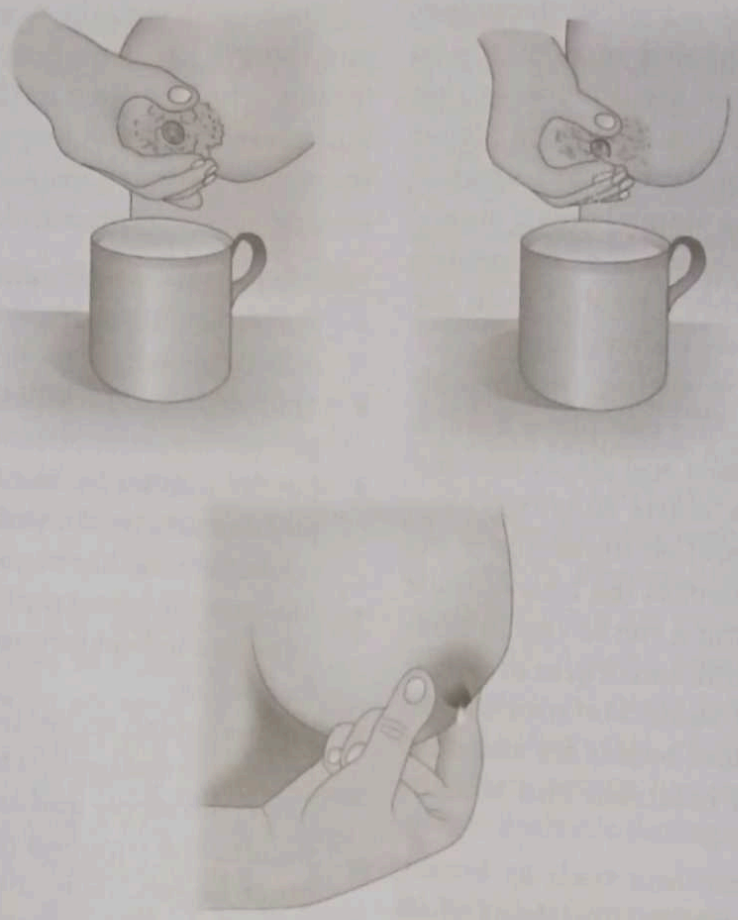

Fig. 1: Manual expression of milk.

- This technique is repeated as the hands rotate around the breast.
- Usually expression of milk from one breast takes around 3–5 minutes after which the milk flow slows, then express from other side and then repeat both sides.
- Avoid squeezing the nipple itself.

KEY MESSAGES

- Mother's Own Milk is the best.
- Minimal enteral nutrition is safe and beneficial with the earlier establishment of feeds, reduced length of stay, and reduced use of parenteral nutrition. Early and rapid enhancement is safe with close monitoring.
- For extremely preterm babies/ELBW, there is no good evidence to support delaying feeds excessively and MEN can be started once baby is hemodynamically stable.
- Stable VLBW babies tolerate early advancement to full feeds.
- In A/REDF, initiate and advance feeding with close monitoring. Optimal duration needed to establish full enteral feeds remains unclear. It should be based on baby's clinical response.

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This information aims to guide physicians in the management of children's kidney diseases and reflects current practice at the Khoo Teck Puat–National University Children's Medical Institute, National University Hospital, Singapore. Considerable care has been taken to ensure that the contents are accurate and current at the time of publication, but the reader is advised to check the drug doses, indications and precautions carefully. The authors are not responsible for any errors which may result from this publication. Application of the information in a particular situation remains the professional responsibility of the practitioner.

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11 Hemolytic-Uremic Syndrome

Lu L, Resontoc LP, Qader MA, Patil M, Liu ID and Yap HK

A. Overview and Definitions

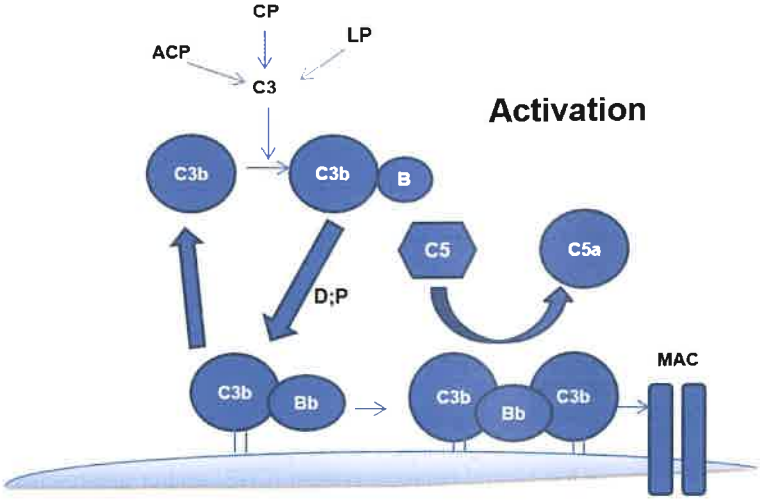
1. Thrombotic microangiopathy (TMA):
   a. Describes a specific pathologic lesion in which abnormalities in the vessel wall of arterioles and capillaries lead to microvascular thrombosis.
   b. TMAs are characterized by:
      - Systemic or intrarenal aggregation of platelets.
      - Thrombocytopenia.
      - Mechanical injury to erythrocytes.
2. Types of TMA:
   a. Hemolytic-uremic syndrome (HUS):
      i. Characterized by the simultaneous occurrence of
         - Acute kidney injury (AKI) of varying severity.
         - Microangiopathic hemolytic anemia (MAHA).
         - Thrombocytopenia.
      ii. Etiology is heterogeneous, which dictates management.
   b. Thrombotic thrombocytopenic purpura (TTP):
      i. Characterized by:
         - MAHA.
         - Thrombocytopenia.
         - Ischemic organ injury with a predilection for involvement of the central nervous system (CNS) and/or gastrointestinal tract.
      ii. In contrast to HUS, there is sparing of the lung and minimal abnormalities of kidney function despite the presence of microthrombi in the kidney.

iii. Caused by severely reduced activity of the von Willebrand factor-cleaving protease ADAMTS13, typically <10%, either due to congenital absence of functional protein or acquired anti-ADAMTS13 antibodies.
iv. Rare in children.

c. Other forms of TMA occur in specific clinical contexts and include:
   i. Disseminated intravascular coagulation (DIC).
   ii. Malignant hypertension.
   iii. Pre-eclampsia/HELLP syndrome (Hemolysis, elevated liver enzymes, low platelets).

B. Complement Regulation

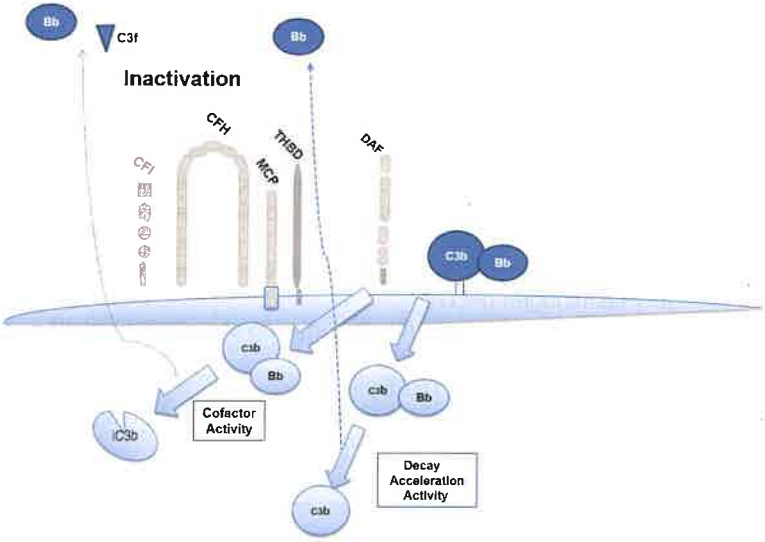
1. Alternative complement pathway (ACP):



a. Positive feedback amplification loop providing rapid antibody-independent defense against microorganisms.

b. Continuously activated through spontaneous hydrolysis of C3 to C3b that combines with complement factor B (CFB), which is then cleaved by factor D to form the C3 convertase (C3bBb).
c. This enzyme complex is stabilized by properdin (P) and is bound to the target covalently by C3b, while Bb is the catalytic serine protease unit.
d. C3b is an opsonin that assists effective phagocytosis and clearance of microbial components and triggers the formation of the terminal products of complement activation, C5a and C5b-9, the membrane attack complex (MAC).
e. The MAC is a pore-like structure that inserts into cell membranes, resulting in cell lysis of invading pathogens.
f. Host cells exposed to sub-lytic doses of the MAC undergo cell activation.

2. Regulation of ACP:
   a. To limit damage to host cells, the alternative pathway is down-regulated by cofactor activity (CA) that leads to permanent inactivation of C3b to iC3b and decay accelerating activity (DAA) that increases the rate of C3 convertase dissociation.



- CA requires both a cofactor protein, complement factor H (CFH) or membrane cofactor protein (MCP, also known as CD46) and a serine protease, and complement factor I (CFI), and is also enhanced by thrombomodulin (THBD).
- DAA is mediated by the decay-accelerating factor (DAF, CD55).

C. Etiological Classification

I. Primary or genetic causes:

1. Complement-mediated HUS:
   a. Gain of function mutations in proteins involved in the alternative pathway:
      - *C3, CFB.*
   b. Loss of function mutations in complement regulatory proteins involved in CA or DAA:
      - *CFH, CD46 (MCP), CFI, THBD.*
   c. Acquired complement dysregulation due to antibodies against CFH (rarely CFB):
      - Often associated with mutations in complement factor H-related proteins (*CFHR1*, *CFHR3*, and *CFHR4*).
2. Mutations in non-complement genes:
   a. Coagulation pathway:
      i. Diacylglycerol kinase epsilon (*DGKE*):
         - DGKE inactivates arachidonic acid-containing diacylglycerols (DAG), an activator of protein kinase C, which promotes thrombosis.
         - Recessive mutations in *DGKE* are associated with HUS.
      ii. Plasminogen (*PLG*):
         - PLG deficiency results in decreased degradation of thrombi.
      iii. Thrombomodulin (*THBD*):
         - THBD enhances CA and is also involved in initiating the protein C anticoagulant pathway.
   b. Cobalamin C (*MMACHC*):
      - Mutations in methylmalonic aciduria and homocystinuria type C (*MMACHC*) gene result in functional Cobalamin

(Vitamin B12) deficiency. Cobalamin is a co-factor for methylmalonyl-CoA mutase and methionine synthase (transforms homocysteine to methionine).

3. Genetic epidemiology:

Complement Abnormality	Prevalence (%)
Complement mediated:	53–64.7
CFH	21.3–25.6
Homozygous	4.2–4.4
Heterozygous	16.8–21.1
CD46	9.2–13.5
Homozygous	5.6
Heterozygous	7.8
CFI	2.6–6.7
C3	3.9–7.8
CFB	0.4–1
THBD	0–7.8
Anti-CFH	3.9–11
Combined (Digenic)	3–3.4
DGKE	7.9
Unknown	27.4–47

   a. Mutations are generally of low penetrance (approximately 50%, with some as low as 20%).
   b. 3.7% of healthy patients will harbor a rare complement gene variant (minor allele frequency <0.1%).

II. Secondary causes:

1. Infection-induced:
   a. Shiga and Shiga-like toxin (STx)-producing bacteria (>90% of HUS):
      i. Shiga toxin-producing enterohemorrhagic *Escherichia coli* (STEC):
         - Increased risk is related to the production of Stx2 (Shiga-like toxin type 2), with or without concomitant Stx1 production.

- Stx2 production is generally (but not universally) associated with these serotypes: O157:H7 (North America and Northwest Europe), O26 (Southern Europe), and O111:H8 (Australia).

ii. *Shigella dysenteriae* type 1 (SD1):
- Entero-invasive, unlike STEC, i.e., hematogenous spread.
- Pathogenesis of HUS is related to a combination of Stx1 and bacterial lipopolysaccharide, and thus, unlike STEC-HUS, patients with *Shigella*-associated HUS can have concomitant DIC.
- The risk of HUS is estimated at 8% during SD1 infection.
- Can uncommonly also be caused by non-SD1 shigellosis (risk of HUS ~1%), e.g., *S. flexneri*, *S. boydii*, and *S. sonnei*.

iii. *Citrobacter freundii*:
- A commensal of the gastrointestinal tract but can infrequently cause diarrhea upon the acquisition of virulence factors.
- Some strains can produce Shiga-like toxins, including *Stx2*.

b. *Streptococcus pneumoniae*:

i. Reported in 5–15% of all childhood cases of HUS and in 40% of non-STEC HUS cases.

ii. The pathogenesis remains unclear, with proposed mechanisms including:
- Classically, neuraminidase is thought to be produced by HUS-inducing pneumococci, leading to cleavage of sialic acid and exposure of the Thomsen–Friedenreich antigen (T-antigen) on erythrocytes, platelets, and glomeruli, resulting in binding and cell lysis by normally circulating anti-T antibodies.
- However, anti-T antibodies are IgM, i.e., low-affinity at body temperature, and erythrocyte desialylation can also occur in pneumococcal disease not progressing to HUS.
- An alternative hypothesis is that pneumococcal neuraminidase may disrupt CFH binding sites, resulting in

unregulated alternative pathway activation similar to complement-mediated primary HUS.
    - Pneumococcal surface proteins may also directly bind and activate PLG.

c. Human immunodeficiency virus.
    - Uncommon with effective anti-retroviral therapy.

d. Other infections:
    - Influenza A (H1N1/H3N2)-associated HUS.
    - *Entamoeba histolytica* intestinal infection.
    - Mycoplasma pneumoniae infection.
    - Hepatitis A (HAV).
    - Hepatitis B (HBV).
    - Cytomegalovirus (CMV).
    - Epstein–Barr virus (EBV).

2. Non-infectious secondary causes of HUS

a. Drug-induced:
    - Calcineurin inhibitors (particularly post-transplantation when high doses are used)
    - Chemotherapy, e.g., cisplatin, mitomycin, bleomycin, and gemcitabine.
    - Other examples include quinine, interferon-beta, oral contraceptives, and bevacizumab (anti-vascular endothelial growth factor).

b. Recent organ transplantation (solid organ and hematopoietic stem cell).

c. Systemic lupus erythematosus (SLE) and anti-phospholipid antibody syndrome:
    - May be associated with decreased ADAMTS13 levels or vWF-cleaving protease inhibitor.
    - May be due to direct endothelial injury with the production of anti-endothelial cell antibodies.

d. Pregnancy:
    - Typically in the post-partum period.
    - Likely to be complement-mediated.

e. Malignancy.

f. May be associated with predisposing mutations in complement genes:

	Complement Abnormality	Prevalence (%)
a. Organ transplantation	Mutations in *CFH* Mutations in *CFI*	15 16
b. Drugs • Cytotoxic agents • Ionizing radiation • CNIs • Oral contraceptives	Rare *CFH* mutations	
c. Pregnancy	Mutations in *CFH* Mutations in *CFI*	20 15

D. Diagnostic Approach

I. Diagnosis of HUS:

1. Clinical features:
   a. Pallor and oliguria, following the onset of infection in typical HUS.
   b. Purpura and bruising are usually mild or absent.
   c. Mild jaundice occurs in about 1/3 of cases.
   d. Hypertension often appears transiently later in the course of AKI.
   e. Non-kidney manifestations:
      i. Central nervous system (CNS) disturbances usually present early in the course of the illness:
         - Irritability, confusion, hallucination, and seizures.
         - Cranial nerve palsy, dysphasia, diplopia, retinal injury, or cortical blindness.
         - Stroke and hemiparesis.
         - Diffuse massive cerebral edema can induce hindbrain compression, leading to brainstem death.
      ii. Gastrointestinal involvement of varying severity, ranging from hemorrhagic colitis to intussusception to bowel necrosis and perforation.
      iii. Mild and transient elevation of liver enzymes, and less often, hepatomegaly.

iv. Pancreatitis: Can lead to overt diabetes mellitus, although this is usually transient.
v. Cardiac ischemia, myocarditis, and pericardial effusion.
vi. Acute respiratory distress syndrome.

2. Laboratory investigations:
   a. Full blood count (FBC) and reticulocytes:
      i. Hemoglobin <10 g/dl (typically <8 g/dl).
      ii. Platelet count <150 × 10^9/l (typically around 40 × 10^9/l).
      iii. Reticulocytosis.
   b. Peripheral blood film for evidence of MAHA:
      i. Red blood cells (RBCs) fragmentation, e.g., schistocytes, helmet cells.
      ii. Decreased platelets.
      iii. If the initial blood film is negative but there is high clinical suspicion, repeat the test 2–3 times to increase sensitivity.
   c. Liver function test and haptoglobin:
      i. Elevated lactate dehydrogenase (LDH) as an index of hemolysis.
      ii. Unconjugated hyperbilirubinemia.
      iii. Decreased serum haptoglobin.
   d. Assessment of kidney function:
      i. Urea, creatinine, sodium, potassium, chloride, bicarbonate, calcium, phosphate, alkaline phosphatase, and uric acid.
         - Raised urea and creatinine with decreased glomerular filtration rate (GFR).
      ii. Urine protein:creatinine ratio or 24-hour urine protein excretion for evidence of significant proteinuria.
      iii. Urinalysis, urine microscopy, and phase contrast study on a fresh urine specimen for evidence of hematuria.
      Note: RBC casts are only occasionally seen.
   e. Coagulopathy screen and direct antiglobulin test:
      i. Normal coagulopathy screen in the absence of fulminant sepsis.
      ii. Direct antiglobulin test (direct Coombs test) to exclude autoimmune hemolytic anemia.
   f. Renal biopsy:
      i. Rarely indicated acutely unless diagnosis is uncertain and the procedure can be performed safely in spite of thrombocytopenia.

ii. There are 3 patterns of TMA that can be observed, i.e., glomerular TMA, cortical necrosis, or arterial/arteriolar-predominant TMA.
   - Arteriolar TMA is seen in complement-mediated HUS but can occur in STEC-HUS.

II. Differentiate infection-induced HUS from other causes:

1. Shiga toxin-associated HUS:
   a. Children >6 months.
      - Greatest risk at age 3–5 years.
   b. STEC-endemic region or *Shigella* endemic region.
      - Risk factors for infection with STEC include salads and vegetables from contaminated surface soil, bathing or swimming in contaminated water, and direct contact with farm animals.
      - Cattle are the main vectors of STEC.
   c. History of diarrhea in the 2 weeks (usually 5–10 days) prior to the onset of HUS.
      - Diarrhea is initially non-bloody, but hematochezia subsequently develops in 60–90% of cases.
   d. Subclinical infection in close contacts.
   e. Investigation for STEC-HUS should be routine in all patients unless another cause is apparent.
      - STEC can often result in mild diarrhea without hematochezia, and approximately 5% of STEC-HUS cases have no prodromal diarrhea.
      - 30% of complement-mediated HUS cases have concurrent diarrhea or gastroenteritis.
2. *Streptococcus pneumoniae*-associated HUS:
   a. Generally restricted to infants and toddlers (median age: 1–2 years old).
   b. Invasive *Streptococcus pneumoniae* infection.
      - Generally pneumonia (70–80%), with 2/3 complicated by empyema,
      - The remaining cases are due to meningitis or acute otitis media, which may be complicated by subdural abscess.
      - 80% of patients are bacteremic.
   c. Onset of HUS usually 1–2 weeks after the onset of illness.

3. When to suspect primary (atypical) HUS:
   a. When any of the following are present:
      i. Severe clinical course without identified underlying cause
      ii. Age <12 months without evidence of a secondary cause.
      iii. Relapse of HUS.
      iv. Suspected previous HUS.
      v. Previous unexplained anemia.
      vi. Asynchronous family history of HUS.
      vii. HUS post-solid organ transplantation.
      viii. Insidious onset: No thrombocytopenia (15%) or anemia (6%), with preserved kidney function initially (17%) in spite of heavy proteinuria.
   b. Suggestive clinical features:
      i. Disorders of complement proteins.
         - Insidious onset precipitated by relatively minor illness such as upper respiratory tract infections or gastroenteritis.
         - Consistent finding at onset is severe hypertension, which can lead to heart failure and encephalopathy.
         - Heavy proteinuria and hematuria occur in those with preserved urine production.
         - Non-kidney manifestations are less frequent than in STEC-induced HUS.
         - Retinal TMA with pain in the eye and visual loss occurs mainly with *CFH* mutations.
         - Hematological relapse is common.
         - Patients presenting in early infancy have a high risk of progression to kidney failure in the 1st episode, and once this has occurred, the MAHA and thrombocytopenia tend to resolve.
      ii. Congenital cobalamin-C mutations:
         - Predominance of neurocognitive symptoms, i.e., hypotonia, lethargy, seizures, anorexia, failure to thrive in the neonatal period, or neuropsychiatric manifestations, and myelopathy in later onset forms.
         - Leukopenia and megaloblastic anemia.

	STEC	*Shigella*	*Streptococcus Pneumoniae*	Primary HUS
Age	1–5 years	Median age 3 years	<2 years	<6 months or >5 years
Onset of HUS	3–10 days after onset of bloody diarrhea (range 1–14 days)	Median time to onset 7 days	7–9 days (range 3–13 days)	Can be rapid but usually subacute (>10 days) following minor illnesses
Non-kidney manifestations	Neurological complications frequent (25%)	Neurological complications frequent	Empyema common; neurological complications rare	Neurological complications rare
Presence of coagulopathy at onset	Nil	Yes if septicemic	Yes if septicemic	Nil
Further investigations	• Stool culture + enhancement and selection techniques such as sorbitol MacConkey agar enriched with tellurite. • Stx immunoassay and PCR for Stx and other virulence vectors • Serological response to locally relevant O serotypes (Serum IgM or saliva IgA/IgM) • Stool culture from household contacts if negative from the patient • STEC excretion in stool lasts only 10–14 days from the onset of diarrhea	• Stool culture	• Chest X-Ray • Blood cultures • Respiratory cultures, pleural tap, OR cerebrospinal fluid cultures • Direct Coombs test (positive in 90%)	Requires • Full investigation for alternative causes of HUS • Investigation for STEC infection, including urine microscopy and culture, as unusual presentations can occur

III.

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III. Exclude TTP and investigate for other causes of HUS:

1. Inherited or acquired ADAMTS13 deficiency:
   a. Plasma vWF protease (ADAMTS13) activity.
      - Measure in the acute phase of illness.
      - Significant if activity <10% of normal.
   b. Test for an inhibitor if ADAMTS13 activity is persistently decreased, even in remission.
      - The presence of an inhibitor suggests an acquired antibody inhibitor.
      - The absence of an inhibitor suggests inherited ADAMTS13 deficiency.
2. Human immunodeficiency virus (HIV) infection:
   - HIV antigen and antibody screen.
3. Systemic lupus erythematosus (SLE) and anti-phospholipid syndrome:
   a. Direct Coombs test.
   b. Serum complements C3 and C4.
   c. Anti-nuclear antibodies (ANA).
   d. Anti-double-stranded DNA antibody.
   e. Lupus anti-coagulant.
   f. Anti-cardiolipin antibody (ACA).
   g. Anti-β2-glycoprotein-1 antibody.
4. Disorders of cobalamin metabolism:
   a. Homocysteine and methylmalonic acid (MMA) (plasma and urine).
      - Vital to test, regardless of clinical suspicion, as potentially treatable with hydroxycobalamin.
   b. Mutation analysis in *MMACHC* gene.
5. Disorders of complement regulation (should be done before commencement of plasma infusions or plasmapheresis):
   a. Serum complement C3:
      - Low C3 in 30% of patients with complement-associated HUS, including 40–60% in patients with anti-CFH antibodies.
      - Not specific, as low C3 levels can transiently occur in STEC-associated and *Streptococcus pneumoniae*-associated HUS, as well as patients with *DGKE* mutations.
   b. CFH autoantibody (enzyme-linked immunosorbent assay).
      - Using standardized assay.

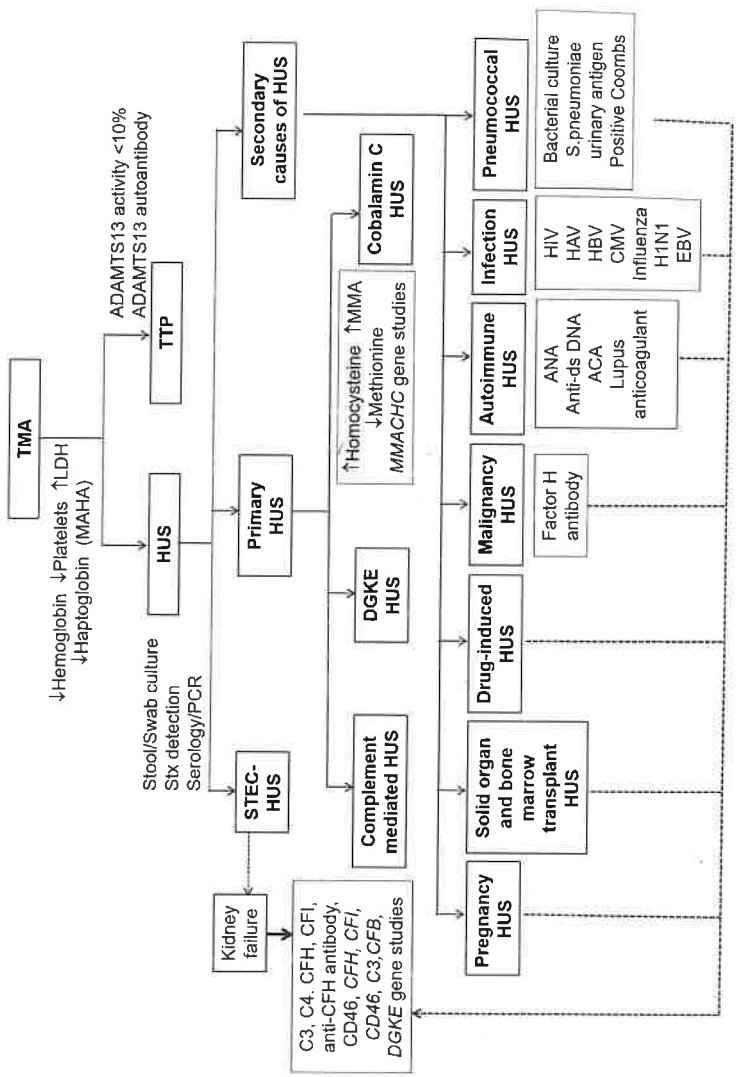
- STEC excretion in stool lasts only 10–14 days from the onset of diarrhea

c. CD46 (MCP) on peripheral blood mononuclear cells by flow cytometry.
   - Time from venesection to analysis should be <4 days because expression of MCP is stable at room temperature for up to this duration.
   - No expression: homozygous deficiency.
   - MFI is 50% in heterozygous individuals.

d. Plasma/serum CFH, CFI, and CFB concentrations (optional):
   - Caution in interpretation as CFH and CFI concentrations may be normal in mutations that do not affect secretion.
   - CFH <60% suggests deficiency.
   - CFI <60% suggests deficiency.
   - Low CFB levels suggest alternate pathway activation.

6. Gene mutation analysis for *CFH*, *CFI*, *CD46*, *CFB*, *CFHR1-5*, *THBD*, *C3*, *DGKE*, and *MMACHC*.
   a. Considerations:
      i. Ensure the chosen genetic test can detect copy number variations in *CFHR1/3* and rearrangements in *CFH-CFHR5*, e.g., by multiplex ligation-dependent probe amplification.
      ii. *DGKE* should also be included in the testing panel when age <2 years.
      iii. Normal levels or expression of CFH, CFI, and CD46 do not rule out the possibility of a normally expressed but functionally impaired mutant.
      iv. Blood for genetic studies should be taken only after 2 weeks following blood transfusions.
   b. Indications:
      i. Start genetic screening without delay if there is clinical suspicion of primary (atypical) HUS.
      ii. Genetic screening is required before kidney transplantation for primary HUS.
         - Not recommended before transplantation for infection-induced HUS unless this diagnosis was uncertain or unproven.

IV. Diagnostic algorithm:



E. Therapy of HUS

I. Supportive therapy:

1. Fluid resuscitation and correction of electrolyte abnormalities and acidosis:
   a. Early and generous volume expansion in STEC-HUS may attenuate the disease course.
      i. Aim for a volume expansion of 10% of working weight by infusion, with 0.9% normal saline of 10 to 15 ml/kg per hour unless there is already evidence of fluid overload.
      ii. Avoid fluid overload >10%.
      iii. Restrict fluids at the 1st sign of hypertension or cardiopulmonary overload.
   b. In all other cases, should aim for clinical euvolemia to maintain tissue perfusion.
   c. Plasma potassium should be closely monitored as it can rise steeply in the presence of red cell fragmentation or with blood transfusion.
   d. Consider dialysis when:
      i. Symptomatic uremia.
      ii. Blood urea ≥30–35 mmol/l.
      iii. Hyperkalemia or acidosis refractory to medical therapy.
      iv. Diuretic-refractory fluid overload.
      v. Need for nutritional support in a child requiring fluid restriction.
2. Transfusion of blood products:
   a. Packed RBC transfusions:
      i. Consider transfusion if hemoglobin is <6 g/dl or hematocrit <18% to avoid cardiovascular and pulmonary compromise.
      ii. Post-transfusion goal of hemoglobin level between 8–9 g/dl is recommended.
         - Restoring the hemoglobin level to normal may cause heart failure, pulmonary edema, and hypertension.
      iii. Transfusion of packed RBCs should be given slowly and cautiously at a total volume of 10 ml/kg over 3–4 hours, with frequent monitoring of the patient's vital signs, which typically produces an increase in hemoglobin of 1 g/dl.

iv. Blood products should be volume-reduced and preferably depleted of leukocytes and platelets to avoid alloimmunization in the event transplantation is subsequently required.
v. Monitor potassium levels closely in the presence of AKI.
vi. If the patient is undergoing hemodialysis, transfusions should be given during dialysis to minimize the risks of hypervolemia and hyperkalemia.
vii. No indication for the routine use of erythropoietin-stimulating agents.

b. Platelet transfusions:
   i. Should be avoided in HUS, to prevent alloimmunization.
      - Limited data available do not support concerns about platelet transfusions fueling further thrombosis.
   ii. Consider platelet transfusions if:
      - Clinical bleeding (rare).
      - Platelet count $<10 \times 10^9$/l (uncommon).
      - Invasive procedures are planned, although increased bleeding complications have not been demonstrated in untransfused HUS patients, e.g., for central venous catheter insertion or peritoneal dialysis catheter insertion.

c. Transfusions in *Streptococcus pneumoniae*-associated HUS:
   i. Consider using washed RBCs to prevent additional administration of anti-T antibodies.
   ii. Consider avoiding plasma infusions (or select plasma with low anti-T titers) due to concerns about driving anti-T-mediated hemolysis.
   iii. These are expert recommendations, although there is limited evidence in support.

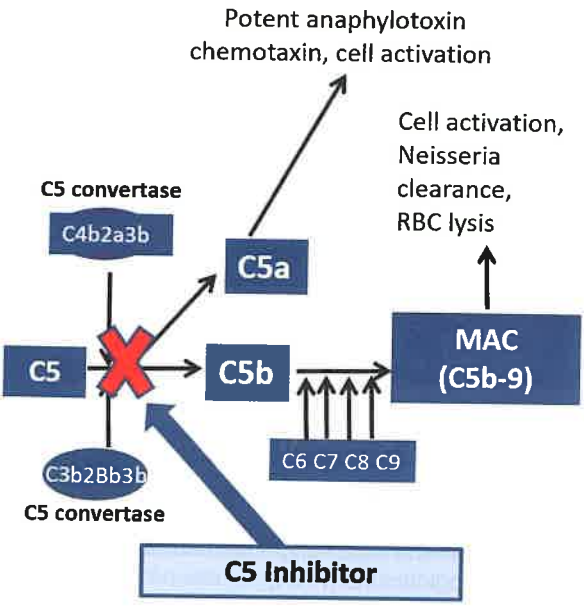
3. Antibiotics and antimotility agents:
   a. Antibiotics and antimotility drugs (such as anticholinergic agents and narcotics) should be avoided in patients with confirmed or suspected STEC-HUS.
      - Antibiotics may injure the bacterial membrane, causing an acute release of pre-formed Shiga toxin, and may induce the bacteriophage carrying the Stx gene, resulting in further toxin production.

b. Antibiotics are indicated in:
   - HUS caused by *Shigella dysenteriae*, as it is enteroinvasive.
   - *Streptococcus pneumoniae*-associated HUS.

4. Other measures:
   a. Antihypertensive therapy.
      i. Calcium channel blockers are recommended as the initial choice of antihypertensive agents in the acute phase of the illness.
      ii. After the acute phase of HUS, angiotensin-converting enzyme (ACE) inhibitors may be given to patients who have renal sequelae.
   b. Neuroprotective measures and anti-seizure treatment for patients with neurological features.
   c. Respiratory support, including pleural tap in *Streptococcus pneumoniae*-associated HUS, as clinically indicated.
   d. Discontinuing nephrotoxic drugs, especially those that may be implicated in drug-associated HUS.
   e. Parenteral nutrition can be given to those who cannot tolerate enteral feeding.

II. C5 inhibitor:

1. Pharmacology:
   a. Mechanism of action:
      - Recombinant humanized monoclonal IgG antibody that inhibits the cleavage of C5 to C5a and C5b, thus preventing the formation of the MAC C5b-9.
      - Eculizumab is currently in clinical use, with plasma $T_{½}$ of 11 days.
      - Ravulizumab is a longer-acting form with plasma $T_{½}$ of 52 days, enabling less frequent dosing.



2. Indications:
   a. First-line therapy for cases of complement-mediated HUS.
      - To be started within 24–48 hours of onset/admission.
   b. Consider a therapeutic trial in STEC-HUS with severe neurological involvement.
      - Give 2 doses and reassess based on clinical response.
      - Recent evidence suggests that eculizumab has no effect on the severity of kidney disease in the acute phase, e.g., duration of kidney replacement therapy, but reduces the incidence of long-term kidney sequelae at 1 year.
   c. No clear evidence for its efficacy in pneumococcal-associated HUS.

3. Efficacy of eculizumab:
   a. Normalization of hematological parameters and improvement of kidney function in 82% and 64%, respectively, at 6 months, and 88% and 76%, respectively, at 2 years.
   b. Effective complement blockade may be assessed through CH_{50} prior to the 2nd dose, aiming for <10%.
      - CH_{50} cannot be measured in those with complete CFH deficiency.
      - Eculizumab blood levels should be measured in complete CFH deficiency, aiming for levels ≥100 μg/ml.
   c. Hematological remission (defined as schistocytes <2%, platelets >100 × 10^9/l, and LDH within normal range) should be attained by 14 days (median 7–8 days), otherwise consider:
      - Patients with severe proteinuria may have reduced eculizumab exposure and be at risk of inadequate complement inhibition.
      - Failure of eculizumab to prevent complement activation may occur in some *C5* variants, which have been described in Japanese and Asian-origin patients with paroxysmal nocturnal hemoglobinuria (PNH).
      - Not effective in patients with primary HUS due to *DGKE* mutations.
4. The main side effect is increased susceptibility to encapsulated bacteria, in particular, *Neisseria meningitidis*, *Streptococcus pneumoniae*, and *Hemophilus influenzae*.
5. Eculizumab maintenance therapy:
   a. The current paradigm is for lifelong therapy to prevent disease recurrence, with doses repeated at 2-weekly intervals.
   b. Dosage regimen (see *G.16. Eculizumab (Anti-C5 Monoclonal Antibody) Therapy for Complement-Mediated Injury*).
   c. Consider stretching the dosing interval based on pre-dose CH_{50}, aiming for ≤10%.
6. Ravulizumab therapy:
   a. Ravulizumab has similar efficacy to eculizumab in both treatment for naïve patients as well as patients maintained on eculizumab.
   b. Dosage regimen for both treatment-naïve patients and patients on eculizumab:

Body Weight	Loading Dose (Day 1)	Maintenance Dose (Start on Day 15)
5–9 kg	600 mg	300 mg every 4 weeks
10–19 kg	600 mg	600 mg every 4 weeks
20–29 kg	900 mg	2100 mg every 8 weeks
30–39 kg	1200 mg	2700 mg every 8 weeks
40–59 kg	2400 mg	3000 mg every 8 weeks
60–99 kg	2700 mg	3300 mg every 8 weeks
≥100 kg	3000 mg	3600 mg every 8 weeks

- For patients on eculizumab, give the loading dose of ravulizumab (Day 1) 14 days after the last dose of eculizumab.

7. Discontinuation may be considered as data emerges regarding the risk, severity, and sequelae of relapses of specific mutations:
   a. In patients with no mutations identified due to a lower risk of relapse.
      - Should be performed after 3–5 years old when viral infections decline in frequency.
   b. In patients with isolated *CD46* mutations due to relatively favorable prognosis, even in the pre-eculizumab era despite a relapsing course.
   c. Frequent monitoring is required for early detection of relapse and re-initiating therapy, including twice-weekly urine dipstick and FBC weekly for 2 months and subsequently monthly.

III. Plasma therapy:

1. Plasma infusion:
   a. Replaces defective complement components and regulators with functional proteins.
   b. In complement-mediated HUS, plasma infusion may be considered in the absence of eculizumab if there is:
      - Mild kidney involvement with conserved urine output.
      - A family member who has responded to plasma infusion.

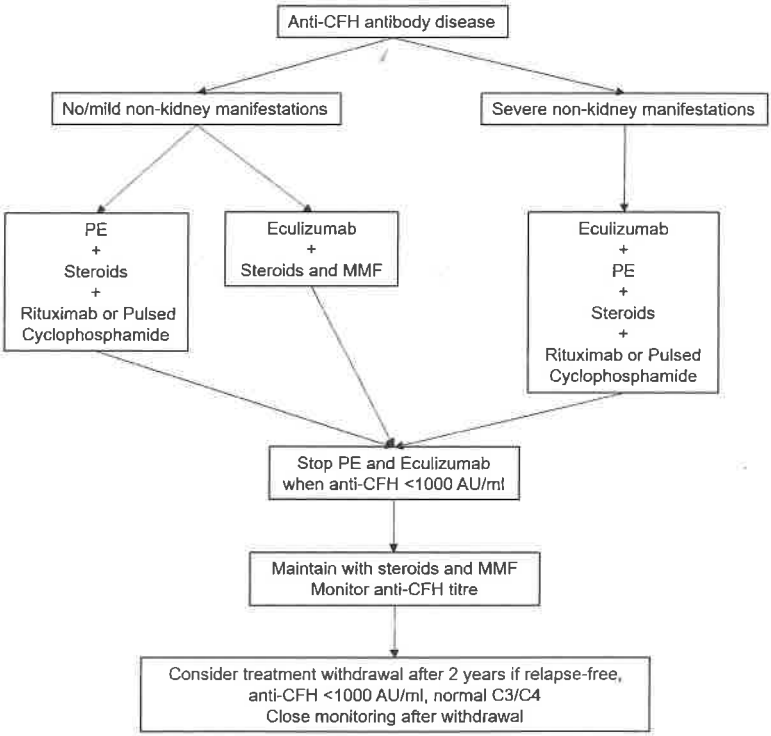
c. Dose:
   - 20–30 ml/kg daily initially.
   - May be limited because of abnormal kidney function and hypertension.

d. If plasma infusion appears successful with the reversal of MAHA, the dose and frequency can be reduced to weekly or biweekly intervals.

2. Plasmapheresis or plasma exchange (PE):
   a. Offers the additional advantage of removal of mutant CFH, CFI, CFB, C3, and CFH autoantibodies while restoring functional complement regulators (except CD46, which is membrane-bound).
   b. Indications:
      i. First-line therapy for severe complement-mediated HUS when eculizumab is not available, except when caused by CD46 mutations.
      ii. Definitive therapy for anti-CFH antibody disease with concomitant immunosuppression.
      iii. Can be considered in STEC-HUS with severe neurological involvement if eculizumab is not available, but there is no strong data to support its efficacy.
   c. PE should be initiated as soon as possible, within 24 hours, to preserve kidney function. In responders, improvement should be observed within 7–10 days.
   d. Prescription:
      i. Schedule: Daily until hematological remission (at least 5 days), then 5 sessions per week for 2 weeks, then 3 sessions per week for 2 weeks.
      ii. Volume of exchange: 1.5 times plasma volume or 60–75 ml/kg in young children.
      iii. Fresh frozen plasma should be used as the replacement.
   e. Maintenance
      i. If genetic screening is pending, plasma infusion of 10–20 ml/kg once every 7–10 days.
      ii. If variants in *CFH*, *CFI*, *C3*, *CFB*, or *THBD*, continue long-term plasma infusions.
      iii. If no variants or variants in *CD46* or *DGKE*, discontinue plasma infusion.

IV. Anti-CFH antibody disease:

1. Principle:
   a. The aim of therapy is to:
      - Block anti-CFH antibody action through PE or eculizumab AND
      - Inhibit anti-CFH antibody production through immunosuppression.
   b. If long-term eculizumab therapy is available, consider eculizumab monotherapy to achieve and maintain remission, especially in the presence of concomitant pathogenic mutations in other complement genes.
2. Treatment algorithm:



3. Dosing:
   a. Eculizumab (see *G.16. Eculizumab (Anti-C5 Monoclonal Antibody) Therapy for Complement-Mediated Injury*).
   b. PE:
      i. Daily exchanges (1.5 times plasma volume, 60 ml/kg) with plasma as the replacement fluid for at least 5 days or until hematological remission is achieved.
      ii. Subsequently, reduce PE to alternate days for 6 sessions, then twice weekly for 6 sessions or until anti-CFH <1000 AU/ml.
   c. Prednisolone:
      i. 1 mg/kg per day × 4 weeks, then
      ii. 1 mg/kg alternate day for 4 weeks, then
      iii. Taper by 0.25 mg/kg every 2 weeks to 0.2–0.3 mg/kg alternate day for 1 year.
   d. Rituximab:
      i. 375 mg/m^2 × 2 doses 1 week apart following hematological remission.
      ii. Avoid PE for 48–72 hours following infusion.
      iii. Consider re-induction with a cyclophosphamide-based regime if relapse occurs while the CD19+ population is still suppressed.
   e. Cyclophosphamide:
      i. IV 500 mg/m^2 for 2–5 doses every 3–4 weeks following hematological remission.
      ii. Avoid PE for 48–72 hours following infusion.
   f. Mycophenolate mofetil (MMF):
      i. 250–375 mg/m^2/dose twice daily if a cyclophosphamide-based induction regime is used.
      ii. 600 mg/m^2/dose twice daily if a non-cyclophosphamide-based induction regime is used.
      iii. Continue for 18–24 months.
4. In patients who do not show hematological remission in 7–10 days:
   a. If on PE, repeat anti-CFH titers and increase exchange volumes/frequency.
   b. If on eculizumab, monitor pre-dose CH50 or eculizumab trough levels and increase eculizumab dosing.
      - Check if the patient carries a C5 variant.
   c. Consider combining PE and eculizumab.

5. Monitor anti-CFH titers for at least the 1st 12–24 months in patients with anti-CFH disease.

V. *Long-term follow-up of primary HUS:*

1. Follow-up monthly and subsequently 3-monthly if stable.
2. Monitor FBC, peripheral blood film, LDH, and haptoglobin:
   a. Hematological relapse is defined as the return of MAHA and thrombocytopenia after these parameters have normalized for at least 2 weeks.
   b. Recurrence of HUS should lead the clinician to consider primary or genetic HUS.
3. Kidney parameters:
   a. Serum creatinine and estimated GFR.
   b. Blood pressure.
   c. Urine protein:creatinine ratio and albumin:creatinine ratio.
4. Other end-organ follow-up depending on the type of non-kidney involvement during the acute phase of disease.

VI. *Treatment summary:*

Disease	Causes	Treatment
Infection	STEC-HUS	• Supportive • No antibiotics • Consider eculizumab for severe neurological manifestations
	Shigella-associated HUS	• Supportive • Antibiotics
	Streptococcus pneumoniae-associated HUS	• Supportive • Antibiotics • Consider avoiding plasma infusions and using washed RBCs for transfusions

(*Continued*)

(*Continued*)

Disease	Causes	Treatment
Disorders of complement regulation	Anti-CFH antibodies	• PE and/or eculizumab/ravulizumab • Immunosuppressive therapy
	Without anti-CFH antibodies	• Eculizumab/Ravulizumab • Consider discontinuation in those with no mutation identified, or *CD46* mutations.
DGKE-associated HUS	Mutations in *DGKE*	• Eculizumab discontinuation, especially after 5 years old • Close monitoring
Cobalamin C-associated HUS	Mutations in *MMACHC*	• Parenteral hydroxocobalamin • Betaine • Methionine • Leucovorin calcium • Carnitine supplements • Genetics referral
Secondary causes	Drugs	• Drug withdrawal • Consider eculizumab if severe and refractory disease. • Consider N-acetylcysteine if there is evidence of TTP.
	SLE ± antiphospholipid syndrome	• Immunosuppressive therapy • PE • ± Anticoagulants
	Viral	• Supportive • Anti-viral therapy • If features are suggestive of viral-triggered complement-mediated HUS, consider eculizumab, or alternatively, PE or plasma infusion.

F. Transplantation

1. Principle:
   a. Determining the risk of relapse and *de novo* disease by screening for complement gene mutations in the recipient and living-related donor, respectively.

b. Perioperative and post-operative prophylaxis will be determined by the risk of relapse.
c. Eculizumab must be available as a rescue therapy, as PE is not effective in recurrent disease in the graft.
d. Avoid endothelial damage.

2. Donor selection:
   a. Consider a living donor if possible.
   b. A living-related donor is suitable if a pathological mutation is found in the recipient and this mutation is absent in the donor.
   c. For deceased donor transplants, avoid prolonged cold ischemia time and non-heart-beating donors.
3. Perioperative and post-operative prophylaxis:
   a. Prophylactic eculizumab in those at high risk of recurrent HUS:
      - Mutations in *CFH*, *CFI*, *C3*, and *CFB*.
      - Previous post-transplant recurrence.
   b. Prophylactic eculizumab can be considered in those at moderate risk:
      - Combined *CD46* mutation.
   c. No prophylaxis is required for those at low risk:
      - Isolated *CD46* mutations.
      - *DGKE* mutations.
      - No mutations identified.
      - Low anti-CFH titer in anti-CFH antibody disease.
   d. Consider adding rituximab to the transplant immunosuppressive regime in those with anti-CFH antibody disease.
   e. If eculizumab is not available, in patients who have responded to PE, consider PE a few hours before transplantation, followed by daily post-transplantation initially, gradually decreasing in frequency.
4. Avoiding endothelial damage:
   a. Antibody-mediated rejection: Avoid transplantation across pre-formed donor-specific antibody or positive cross-match.
   b. Infections: CMV prophylaxis.
   c. Immunosuppression: Careful monitoring of CNI levels to avoid overdosing with consideration for low-dose regimes.
      - CNI-free mTORi-based regimes should be avoided.
   d. Hypertension: Strict blood pressure control.

5. Role of liver transplantation:
   a. Many (but not all) complement components and regulatory proteins are synthesized in the liver.
   b. Combined liver-kidney transplant or pre-emptive liver transplant is potentially curative in those with high-risk mutations, i.e., *CFH*, *CFI*, *C3*, *CFB*, or otherwise at high risk of recurrence.
   c. May be an alternative to long-term post-kidney transplant eculizumab/ravulizumab prophylaxis, but there is currently limited experience.
   d. These patients will still require perioperative prophylaxis (possibly including PE), without which perioperative mortality may be 100%.

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ಡಾ॥ ರಣಜಿತ ಬೀರಣ್ಣ ನಾಯ್ಕ ಕೆಂಚನ್
ಹಿರೇಗುತ್ತಿ

ಹೃದಯ ಸಂಗ್ರಾಮ

ಸಪ್ನ

ಡಾ॥ ರಣಜಿತ ಬೀರಣ್ಣ ನಾಯ್ಕ ಕೆಂಚನ್

ಮೂಲತಃ ಉತ್ತರ ಕನ್ನಡ ಜಿಲ್ಲೆಯ ಕುಮಟಾ ತಾಲೂಕಿನ ಹಿರೇಗುತ್ತಿಯವರಾದ ಇವರು ವೃತ್ತಿಯಲ್ಲಿ ಹೃದ್ರೋಗ ಶಸ್ತ್ರಕ್ರಿಯೆ ತಜ್ಞರಾಗಿದ್ದಾರೆ. ಆರಂಭಿಕ ಹಂತದಲ್ಲಿ ಅಂಕಣ ಬರವಣಿಗೆಯಲ್ಲಿ ಗುರುತಿಸಿಕೊಂಡಿದ್ದ ಇವರು "ಕರಾವಳಿ ಮುಂಜಾವು" ಪತ್ರಿಕೆಗೆ 2016ರಿಂದ 2020ರ ವರೆಗೆ ನಿರಂತರವಾಗಿ ಪ್ರತಿ ವಾರ ಆರೋಗ್ಯ ಅಂಕಣ ಬರೆದಿದ್ದರು. ಅವರ ಮೊದಲ ಕೃತಿಯಾದ "ವೈದ್ಯ ವಿಜ್ಞಾನ" ಕ್ಕೆ ಕರ್ನಾಟಕ ವಿಜ್ಞಾನ ಮತ್ತು ತಂತ್ರಜ್ಞಾನ ಅಕಾಡೆಮಿಯ ಶ್ರೇಷ್ಠ ಪುಸ್ತಕ ಪ್ರಶಸ್ತಿ ದೊರಕಿದೆ.

ಆರೋಗ್ಯ, ಹೃದ್ರೋಗ, ರಕ್ತದಾನ, ಹಿಮೋಫಿಲಿಯಾ, ಮಧುಮೇಹ ಕುರಿತು ಅರಿವು, ಪರಿಸರ ರಕ್ಷಣೆ ಮುಂತಾದ ಸಾಮಾಜಿಕ ಕಾರ್ಯಗಳಲ್ಲಿ ತೊಡಗಿಸಿಕೊಂಡಿದ್ದಾರೆ. ಭಾರತ, ವೈದ್ಯಕೀಯ ಸಂಘ ಸೇರಿದಂತೆ ಹಲವು ಸಂಘ-ಸಂಸ್ಥೆಗಳ ಸದಸ್ಯರಾಗಿದ್ದು, ಅನೇಕ ರಾಷ್ಟ್ರೀಯ-ಅಂತರಾಷ್ಟ್ರೀಯ ಸಭೆ, ಸಮಾವೇಶಗಳಲ್ಲಿ ವೈದ್ಯಕೀಯ ವಿಚಾರ ಗಳನ್ನು ಮಂಡಿಸಿರುತ್ತಾರೆ. ವಲಯ ಮಟ್ಟದ ಕ್ರಿಕೆಟಿಗರೂ ಸಹ ಆಗಿದ್ದ ಇವರದ್ದು ಬಹುಮುಖ ಪ್ರತಿಭೆ. ಜನಸಾಮಾನ್ಯರಿಗೆ ವೈದ್ಯಕೀಯ ಸಂಬಂಧಿ ವಿಚಾರಗಳು ಅರ್ಥ ವಾದಾಗ ಮಾತ್ರವೇ ಚಿಕಿತ್ಸೆ ಸಫಲವಾಗಲು ಸಾಧ್ಯ ಎಂಬ ಬಲವಾದ ನಂಬಿಕೆ ಹೊಂದಿರುವ ಇವರು ವಿಜ್ಞಾನದ ಕಾರ್ಯದಲ್ಲಿ, ಸಾಹಿತ್ಯ ಕೃಷಿಯಲ್ಲಿ ತೊಡಗಿಸಿ ಕೊಂಡಿದ್ದಾರೆ. ಪ್ರಜಾವಾಣಿ ಬಳಗ 2020ರಲ್ಲಿ ಇವರನ್ನು 'ಯುವ ಸಾಧಕ'ರಾಗಿ ಗುರುತಿಸಿ ಸನ್ಮಾನಿಸಿದೆ.

ಇವರು ಪ್ರಸ್ತುತ ಬೆಳಗಾವಿಯ ಕೆ.ಎಲ್.ಇ.ಎಸ್. ಡಾ॥ ಪ್ರಭಾಕರ ಕೋರೆ ಆಸ್ಪತ್ರೆಯಲ್ಲಿ ಹೃದ್ರೋಗ ಶಸ್ತ್ರಕ್ರಿಯೆ ತಜ್ಞರಾಗಿ ಹಾಗೂ ಜವಾಹರಲಾಲ್ ನೆಹರು ವೈದ್ಯಕೀಯ ಕಾಲೇಜಿನಲ್ಲಿ ಸಹಾಯಕ ಪ್ರಾಧ್ಯಾಪಕರಾಗಿ ಕಾರ್ಯನಿರ್ವಹಿಸುತ್ತಿದ್ದಾರೆ.

ಇನ್ನಿತರೆ ಕೃತಿಗಳು

* ವೈದ್ಯ ವಿಜ್ಞಾನ (2018)
* ವೈದ್ಯ ದರ್ಪಣ (2019)
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ಅರ್ಪಣೆ

ಬದುಕಿನಲ್ಲಿ ಇಚ್ಛಾಶಕ್ತಿ, ಸಾಧಿಸುವ ಹಂಬಲವನ್ನು
ಮನಮುಟ್ಟುವಂತೆ ಸ್ವಂತ ಜೀವನದ ಮೂಲಕವೇ
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ನಲ್ನುಡಿ

ಹೃದ್ರೋಗ ಚಿಕಿತ್ಸೆಯ ಮೈಲುಗಲ್ಲುಗಳ ಐತಿಹಾಸಿಕ ಪಯಣ...

ಮಾನವನು ಅತ್ಯಂತ ಸಂಕೀರ್ಣ ರಚನೆ ಹಾಗೂ ಕ್ರಿಯೆಗಳಿಂದ ಕೂಡಿದ ಸೃಷ್ಟಿಯ ಅದ್ಭುತಗಳಲ್ಲೇ ಚಿಗ್ರಗಣ್ಯವೆನಿಸಿದ್ದಾನೆ. ಶಾರೀರಿಕ ಸಂಕೀರ್ಣತೆ ಒಂದು ಕಡೆಯಾದರೆ ಮಾನಸಿಕ, ಸಾಮಾಜಿಕ, ಆಧ್ಯಾತ್ಮಿಕ, ಭಾವನಾತ್ಮಕ ಹೀಗೆ ಹಲವು ಆಯಾಮಗಳಿಗೂ ವಿಸ್ತರಿಸಿದೆ. ಇಷ್ಟೆಲ್ಲಾ ವೈವಿಧ್ಯತೆಗಳಿಂದ ಕೂಡಿದ ಮಾನವ ತನ್ನ ಅಪರಿಮಿತ ಬುದ್ಧಿಶಕ್ತಿಯಿಂದಾಗಿ, ಅನೇಕಾನೇಕ ಆವಿಷ್ಕಾರ ಹಾಗೂ ತಂತ್ರಜ್ಞಾನಗಳಿಂದ ವಿಶ್ವದ ಬಹು ವಿಸ್ಮಯಗಳಿಗೆ ಕಾರಣೀಭೂತನಾಗಿ 'ಸೃಷ್ಟಿಯ ಮುಕುಟ'ನೆನಿಸಿದ್ದಾನೆ. ಸಾಧಿಸಿದ್ದು ಬಹಳಷ್ಟಿದೆಯಾದರೂ, ಸಾಧಿಸಬೇಕಾದುದು ಸಾಗರದಷ್ಟಿದೆ. ಮಾನವ ಜನಾಂಗಕ್ಕೆ ನೆನಪಿರಬಹುದಾದ ಸಾವಿರಾರು ವರ್ಷಗಳ ಈ ಅವಧಿಯೊಳಗೆ ಕೈಗೊಂಡ ವಿವಿಧ ಸಾಧನೆ–ಸಂಶೋಧನೆಗಳು ಜೀವನದ ದಿಕ್ಕನ್ನೇ ಬದಲಿಸಿ ಹೊಸ ಮನ್ವಂತರದ ಸೃಷ್ಟಿಗೆ ಕಾರಣವಾಗಿದೆ. ಇಂತಹ ಐತಿಹಾಸಕ ಪಯಣವು ನಮ್ಮ ವರ್ತಮಾನ–ಭವಿಷ್ಯಗಳ ಹೋರಾಟ–ಹಾರಾಟ ಗಳಿಗೆ ಮುನ್ನುಡಿಯಾಗಬಹುದು. 12ನೇ ಶತಮಾನದ ಶರಣ ಅಲ್ಲಮಪ್ರಭುಗಳು ಈ ಕುರಿತಾಗಿ ಹೀಗೆ ಹೇಳುತ್ತಾರೆ–"ಹಿಂದಣ ಹೆಜ್ಜೆಯ ನೋಡಿ ಕಂಡಲ್ಲದೆ ನಿಂದ ಹೆಜ್ಜೆಯನರಿಯಬಾರದು."

ವರ್ತಮಾನದ ಮಾನವ ಅನೇಕ ವೈಜ್ಞಾನಿಕ ಹಾಗೂ ತಂತ್ರಜ್ಞಾನಗಳ ಸಹಾಯದಿಂದ ಬದುಕನ್ನು ಸುಲಭಸಾಧ್ಯವಾಗಿಸಿದ್ದಾನೆ. ಅದರೊಟ್ಟಿಗೆ ಪ್ರಕೃತಿಯ ವಿರುದ್ಧವಾಗಿ ಚಲಿಸಿ, ಪ್ರಕೃತಿಯನ್ನೇ ಹಿಮ್ಮೆಟ್ಟಿಸಿ, ಗೆಲ್ಲಬೇಕೆಂಬ ಹುಚ್ಚು, ಅವನ ಬದುಕನ್ನೇ ನಾಶಪಡಿಸುವ ಹಂತವನ್ನು ತಲುಪಿದೆ. ಈ ಎಲ್ಲ ಆಗು–ಹೋಗುಗಳ

ಸಂದರ್ಭದಲ್ಲಿ ಒತ್ತಡದ ಬದುಕಿಗೆ ದಾಸನಾಗಿ, ತನ್ನ ಅಮೂಲ್ಯ "ಆರೋಗ್ಯ" ವನ್ನು ಕಳೆದುಕೊಂಡು, ರೋಗದ ಗೂಡಾಗಿದ್ದಾನೆ. ಅಂತೆಯೇ ಇಂದಿನ ವೈದ್ಯಕೀಯ ವಿಜ್ಞಾನದ ಆರೋಗ್ಯದ ಬದಲಾಗಿ ವಿವಿಧ ರೋಗಗಳ ಅಧ್ಯಯನಕ್ಕೆ ಪ್ರಾಶಸ್ತ್ಯ ನೀಡುವಂತಾಗಿದೆ. ರೋಗಗಳು ಎಷ್ಟು ವಿಧವಾಗಿ, ವಿಭಿನ್ನವಾಗಿ ತಲೆಯೆತ್ತುತ್ತಿವೆ ಎಂದರೆ 'ಆರೋಗ್ಯ'ವೆಂಬುದು ಮರಿಚಿಕೆಯಾಗುತ್ತಿದೆ. ಜಾಗತಿಕ ಮಟ್ಟದಲ್ಲಿ ಮನುಷ್ಯನು ಬಳಲುತ್ತಿರುವ ರೋಗಗಳು, ಅದರಿಂದ ಸಂಭವಿಸುವ ಸಾವುಗಳಲ್ಲಿ ಹೃದಯ ಸಂಬಂಧಿ ಕಾಯಿಲೆಗಳದ್ದೇ ಮೇಲುಗೈಯಾಗಿದೆ. ಅಂತೆಯೇ ಹೃದ್ರೋಗವು ವಿಶ್ವದಲ್ಲಿ ಸಂಭವಿಸುವ ಸಾವು–ನೋವುಗಳ ಬಹುತೇಕ ಅರ್ಧಪಾಲನ್ನು ಹೊಂದುತ್ತಿದೆ. ಹೃದಯ ರೋಗವು ಕೇವಲ ಒಂದು ರೋಗ ವಾಗದೇ ಅದೊಂದು ರೋಗಗಳ ಸಂಕೀರ್ಣ (Constellation of Diseases) ಎಂದರೆ ತಪ್ಪಲ್ಲ. ಹೀಗಾಗಿ ಯಾವುದೇ ವ್ಯಕ್ತಿಗೆ ಹೃದ್ರೋಗವಿರುವುದು ದೃಢಪಟ್ಟರೆ ಆ ವ್ಯಕ್ತಿಯ, ಆ ಮನೆಯ ಚಿತ್ರಣವೇ ಬದಲಾಗುತ್ತದೆ.

ಇಷ್ಟೆಲ್ಲಾ ಆಲೋಚನೆ–ಆವಿಷ್ಕಾರಗಳ ಕರ್ತೃವಾದ ಮನುಷ್ಯ ಈ ಸಂಕೀರ್ಣ ರೋಗ ಸಂಕುಲವಾದ 'ಹೃದ್ರೋಗಕ್ಕೆ' ಮದ್ದು ಕಂಡುಹಿಡಿದಿಲ್ಲವೇ? ಖಂಡಿತವಾಗಿಯೂ ಕಂಡುಹಿಡಿದಿರುವುದು ಮಾತ್ರವಲ್ಲ ಹೃದ್ರೋಗದ ಚಿಕಿತ್ಸೆಯ ದಿಕ್ಕನ್ನೇ ಬದಲಿಸಿ ನಿರಂತರ ಚಿಂತನೆ–ಆವಿಷ್ಕಾರಗಳಿಂದ ವೈದ್ಯ ವಿಜ್ಞಾನವು ಪ್ರಗತಿಯ ಪಥದಲ್ಲಿ ಸಾಗಿದೆ. ಅದಕ್ಕಾಗಿ ಅನೇಕ ವೈದ್ಯ ವಿಜ್ಞಾನಿಗಳು ತಮ್ಮ ಜೀವನವನ್ನೇ ಸಮರ್ಪಿಸಿಕೊಂಡಿದ್ದಾರೆ. ಆದರೆ ಅವರು ಈ ಸಾಧನೆಗೆ ಕ್ರಮಿಸಿದ ಹಾದಿ ಹೂವಿನ ಹಾಸಿಗೆಯಾಗಿರಲಿಲ್ಲ. ಬದಲಿಗೆ ಹಲವು ಆಕಸ್ಮಿಕಗಳ, ಆವಿಷ್ಕಾರ ಗಳ, ದುರಂತಗಳ, ತ್ಯಾಗಗಳ, ಪರಿಶ್ರಮಗಳ, ಸಂಶೋಧನೆಗಳ, ಧೃತಿಗೆಡದ, ಭಾವನಾತ್ಮಕ ಮನಸ್ಸುಗಳ ಸಂಗಮವಾಗಿದೆ. ಒಂದು ರೀತಿಯಲ್ಲಿ ಹೃದ್ರೋಗ ಪೀಡಿತ ಹೃದಯಗಳೊಂದಿಗೆ, ಆ ನೋವಿಗೆ ಸ್ಪಂದಿಸುವ, ಮಿಡಿಯುವ ಹೃದಯಗಳ "ಸಂಗ್ರಾಮ" (Battle) ಇದ್ದಂತೆ. ಇಂತಹ ಅನೇಕ ವಿಸ್ಮಯಕಾರಿ, ಐತಿಹಾಸಿಕ ಸಂಗ್ರಾಮ ನಡೆಸಿದ ಹೃದಯಗಳ ಕಥೆಗಳ ಮಾಲಿಕೆಯೆ, ನಿಮ್ಮ ಕೈಯಲ್ಲಿರುವ ಕೃತಿ "ಹೃದಯ ಸಂಗ್ರಾಮ". ಈ ಹೃದಯ ಸಂಗ್ರಾಮಗಳನ್ನು ನಮಗಾಗಿ ಕಟ್ಟಿಕೊಟ್ಟವರು ವೃತ್ತಿಯಲ್ಲಿ ಹೃದಯ ಶಸ್ತ್ರಚಿಕಿತ್ಸಕರಾಗಿ ನಿರಂತರವಾಗಿ ಹೃದ್ರೋಗದೊಂದಿಗೆ ಸೆಣೆಸಾಡುತ್ತಿರುವ ಡಾ॥ ರಣಜಿತ್ ಬೀರಣ್ಣ ನಾಯಕರವರು.

ಡಾ॥ ರಣಜಿತ್ ನಾಯಕರವರು ನಮ್ಮ ದಿನಮಾನದ ಯುವ ವೈದ್ಯ ಲೇಖಕರಾಗಿ, ಕನ್ನಡ ವೈದ್ಯ ಸಾಹಿತ್ಯಕ್ಕೆ ತಮ್ಮ ಅಮೂಲ್ಯ ಸೇವೆಯನ್ನು ಸಲ್ಲಿಸುತ್ತಿದ್ದಾರೆ. ಚಿಕ್ಕ ವಯಸ್ಸಿನಲ್ಲಿಯ ಸಂಸ್ಕಾರ, ಅವರ ಕುಟುಂಬದ ಪ್ರೋತ್ಸಾಹ,

ಶಿಕ್ಷಕರ ಮಾರ್ಗದರ್ಶನ, ತಾನು ಕಲಿತ ವಿದ್ಯೆಯನ್ನು ಜನಸಾಮಾನ್ಯರಿಗೂ ತಲುಪಿಸಬೇಕೆಂಬ ಹಂಬಲಗಳು ಮೇಳೈಸಿ, ತಮ್ಮ ನಿರಂತರ ಸವಾಲುಗಳ ವೃತ್ತಿಯಾದ ಹೃದಯ ಶಸ್ತ್ರಚಿಕಿತ್ಸೆಯೊಂದಿಗೆ ಸಾಹಿತ್ಯದ ಅಭಿರುಚಿಯನ್ನು ಜಾಗೃತವಾಗಿಟ್ಟು, ಆ ನಿಟ್ಟಿನಲ್ಲಿ ಪ್ರಯತ್ನಪೂರ್ವಕವಾಗಿ ಮುತುವರ್ಜಿಯಿಂದ ಶ್ರಮವಹಿಸಿ ಈಗಾಗಲೇ ಆರು ಮೌಲಿಕ ಕೃತಿಗಳನ್ನು ರಚಿಸಿರುವ ಇವರು ನಮ್ಮ ಯುವ ವೈದ್ಯರಿಗೆ ಮಾದರಿಯಾಗಿದ್ದಾರೆ. 'ಹೃದಯ ಸಂಗ್ರಾಮ' ಅವರ ಏಳನೆಯ ಕೃತಿಯಾಗಿದೆ. ಸ್ವತಃ ಹೃದ್ರೋಗ ಚಿಕಿತ್ಸೆಯ ಪರಿಣಿತರಾಗಿ ಹೃದಯ ಸಂಬಂಧಿ ರೋಗಗಳ ಚಿಕಿತ್ಸೆ, ಅದನ್ನು ತಡೆಗಟ್ಟುವ ವಿಧಾನಗಳ ಕುರಿತಾಗಿ ಜನಸಾಮಾನ್ಯ ರನ್ನು ದೃಷ್ಟಿಯಲ್ಲಿರಿಸಿಕೊಂಡು ಕನ್ನಡದಲ್ಲಿ ಅನೇಕ ಅಂಕಣಗಳನ್ನು ಹಾಗೂ ಪುಸ್ತಕಗಳನ್ನು ರಚಿಸಿರುವ ಇವರು ಪ್ರಸ್ತುತ ಕೃತಿಯಲ್ಲಿ ಹೃದ್ರೋಗ ಚಿಕಿತ್ಸೆ ಹಾಗೂ ಶಸ್ತ್ರಕ್ರಿಯೆಗಳ ಆವಿಷ್ಕಾರ, ಸಂಶೋಧನೆಗಳ ಮೈಲುಗಲ್ಲುಗಳನ್ನು, ಅದರ ಸಾಹಸ ಗಾಥೆಗಳನ್ನು ಬಿಚ್ಚಿಡುವ ಪ್ರಯತ್ನವನ್ನು ಸಮರ್ಥವಾಗಿ ಮಾಡಿದ್ದಾರೆ.

ಡಾ॥ ರಣಜಿತ್ ಅವರೇ ಹೇಳಿಕೊಂಡಂತೆ, 'ಹೃದಯ ಸಂಗ್ರಾಮ' ಎಂಬ ಕೃತಿಯಲ್ಲಿ ಹೃದ್ರೋಗಕ್ಕೆ ಶಾಶ್ವತ ಪರಿಹಾರ ಕೈಗೊಳ್ಳಬೇಕೆಂಬ ದಿಟ್ಟ ಹೃದಯಗಳ ಸಾಹಸಗಾಥೆಯನ್ನು ಕಟ್ಟಿಕೊಟ್ಟು, ಹೃದಯ ಚಿಕಿತ್ಸಕರು–ಶಸ್ತ್ರಚಿಕಿತ್ಸಕರು ಪಟ್ಟ ಪ್ರಯಾಸ, ಅವರೊಂದಿಗೆ ಸಹಕರಿಸಿದ ವ್ಯಕ್ತಿಗಳ, ಚಿಕಿತ್ಸೆಗೆ ಒಪ್ಪಿದ ರೋಗಿಗಳ, ಕೆಲವೊಮ್ಮೆ ಚಿಕಿತ್ಸೆ ಫಲಿಸದೆ ಪ್ರಾಣತೆತ್ತವರ ಕಥೆಗಳ ಸಾರವೇ ಅಡಗಿದೆ.

ಜಗತ್ತಿನ ಮೊಟ್ಟಮೊದಲ ಹೃದಯ ಶಸ್ತ್ರಚಿಕಿತ್ಸೆಯಿಂದ ಪ್ರಾರಂಭವಾಗುವ ಈ ಕೃತಿಯ ಮೊದಲ ಅಧ್ಯಾಯ–'ಅಸಾಧ್ಯವ ಮೀರಿದ ಅಸಂಪ್ರದಾಯಕ ನಡೆ' ಒಂದು ವಿಸ್ಮಯ ಜಗತ್ತಿಗೆ ನಮ್ಮನ್ನು ಕೊಂಡೊಯ್ಯುತ್ತದೆ. ಜಗತ್ತಿನ ಮೊಟ್ಟಮೊದಲ ತೆರೆದ ಹೃದಯ ಶಸ್ತ್ರಚಿಕಿತ್ಸೆ ನಡೆದ ಬಗೆ ಹಾಗೂ ಅದನ್ನು ಕೈಗೊಂಡ ವೈದ್ಯನ ಅಸಾಮಾನ್ಯ ದಿಟ್ಟತನ ಓದುಗರ ಗಮನವನ್ನು ಹಿಡಿದಿಡುತ್ತದೆ. 19ನೇ ಶತಮಾನದ ಕೊನೆಯ ಭಾಗದಲ್ಲಿ, ಆವರೆಗೂ ವೈದ್ಯ ವಿಜ್ಞಾನ ನಂಬಿದ್ದ "ಹೃದಯ ಶಸ್ತ್ರಚಿಕಿತ್ಸೆಯ ನೈಸರ್ಗಿಕ ಅಲಭ್ಯ ಚಿಕಿತ್ಸೆ, ಅದರ ಕುರಿತಾದ ಯಾವುದೇ ಆವಿಷ್ಕಾರಗಳು ಸಫಲವಾಗಲು ಸಾಧ್ಯವೇ ಇಲ್ಲ" ಎಂಬ ಸ್ಥಾಪಿತ ಸಿದ್ಧಾಂತವನ್ನು ಧಿಕ್ಕರಿಸಿ ನಡೆದ ಧೀರ ವೈದ್ಯ ವಿಜ್ಞಾನಿಗಳು ತಮ್ಮ ಅಮೋಘ ಆಲೋಚನಾ ಸಾಮರ್ಥ್ಯದಿಂದ ಹೃದಯ ಶಸ್ತ್ರಚಿಕಿತ್ಸೆಯ ಮಾರ್ಗವನ್ನೇ ಬದಲಿಸಿ ಸಾವಿರಾರು–ಲಕ್ಷಾಂತರ ರೋಗಿಗಳಿಗೆ ದೈವ ಸಮಾನರಾದ ಕಥೆಗಳೊಂದಿಗೆ ಕೃತಿಯು ಓದುಗರಲ್ಲಿ ಆಸಕ್ತಿಯ ತೀವ್ರತೆಯನ್ನು ಹೆಚ್ಚಿಸುತ್ತದೆ.

"Necessity is the mother of Invention"–'ಅವಶ್ಯಕತೆಯೇ ಎಲ್ಲಾ

ಆವಿಷ್ಕಾರಗಳ ತಾಯಿ' ಎಂಬ ಮಾತಿನಂತೆ, ತಮ್ಮ ಸುತ್ತಲೂ ಬಳಲುತ್ತಿರುವ ರೋಗಿಗಳ ಆ ನೋವಿಗೆ ಸ್ಪಂದಿಸುವ ವೈದ್ಯ ವಿಜ್ಞಾನಿಗಳ ರೋಚಕ ಸಂಶೋಧನೆಗಳನ್ನು ಒಂದೊಂದಾಗಿ ದಾಖಲಿಸುತ್ತಾ ಸಾಗುವ ಕೃತಿಯು, ಹೃದಯ ಶಸ್ತ್ರಚಿಕಿತ್ಸೆಯ ಮೈಲುಗಲ್ಲುಗಳ ಸ್ಥೂಲ ವಿವರಗಳನ್ನು ಎಳೆ–ಎಳೆಯಾಗಿ ಬಿಡಿಸುತ್ತಾ ಸಾಗುತ್ತದೆ. ಕೆಲವೊಮ್ಮೆ ಹಿರಿಯ ವೈದ್ಯರ ಶಿಷ್ಯರ ಆವಿಷ್ಕಾರಗಳು ಹೇಗೆ ಹೊಸ ಪದ್ಧತಿಯ ಶಸ್ತ್ರಚಿಕಿತ್ಸೆ ರೂಪುಗೊಳ್ಳಲು ಸಹಕಾರಿಯಾದವು ಎಂಬ ಅನೇಕ ವಿಸ್ಮಯಕಾರಿ ಸಂಗತಿಗಳನ್ನು ದಾಖಲಿಸಲಾಗಿದೆ. ಅದಕ್ಕಾಗಿ ಅನೇಕ ವೈದ್ಯರು ತಮ್ಮ ಜೀವನವನ್ನೇ ಬಲಿಕೊಡಬೇಕಾದ ದುರಂತಗಳು ನಮ್ಮ ಕಣ್ಣಾಲಿಗಳನ್ನು ಒದ್ದೆಯಾಗಿಸುತ್ತವೆ. ಹೃದಯ ಶಸ್ತ್ರಚಿಕಿತ್ಸೆಯಲ್ಲಿ ಹೊಸ ಮನ್ವಂತರವನ್ನು ಸೃಷ್ಟಿಸಿದ ಆವಿಷ್ಕಾರ "ಕೃತಕ ಹೃದಯ ಶ್ವಾಸಕೋಶ ಯಂತ್ರ". ಅದನ್ನು ಕಂಡುಹಿಡಿದ ಅಪೂರ್ವ ಸನ್ನಿವೇಶ ಹಾಗೂ ಆ ಆವಿಷ್ಕಾರದಿಂದ ಪ್ರಸ್ತುತ ಶೇ. 80 ಹೃದಯ ಶಸ್ತ್ರಚಿಕಿತ್ಸೆಗಳು ನಡೆಯಲು ಸಾಧ್ಯವಾಗಿದೆ ಎಂಬ ವಿಸ್ಮಯಕಾರಿ ಅಂಶವು ನಮ್ಮನ್ನು ರೋಮಾಂಚನಗೊಳಿಸುವುದಂತೂ ಸತ್ಯ. ಈ ಯಂತ್ರವನ್ನು ಬಳಸಿ ನಡೆಸಿದ ಐತಿಹಾಸಿಕ ಹೃದಯ ಶಸ್ತ್ರಚಿಕಿತ್ಸೆ ಹಾಗೂ ಅದನ್ನು ಸ್ವೀಕರಿಸಿದ ದಿಟ್ಟ ರೋಗಿ ಸಿಸಿಲಿಯಾ ಮುಂದೆ 'ಹೃದಯದ ರಾಣಿ' (Queen of Hearts) ಎಂಬ ಅಭಿಧಾನದೊಂದಿಗೆ ಮಕ್ಕಳ ಹೃದಯ ಶಸ್ತ್ರಚಿಕಿತ್ಸೆಯ ರಾಯಭಾರಿಯಾದ ಬಗೆಯಂತೂ ಓದುಗರಿಗೆ ವಿಸ್ಮಯ–ಆನಂದವನ್ನು ನೀಡುತ್ತದೆಂಬುದರಲ್ಲಿ ಎರಡು ಮಾತಿಲ್ಲ.

ಹೃದಯ ಶಸ್ತ್ರಚಿಕಿತ್ಸೆಯ ಸಂಕೀರ್ಣತೆ, ಅದರ ಅಭಿವೃದ್ಧಿ, ವಿವಿಧ ಭಾಗಗಳ ಶಸ್ತ್ರಚಿಕಿತ್ಸೆಗಳು, ಅದಕ್ಕಾಗಿ ತೆಗೆದುಕೊಂಡ ಅಪಾಯಗಳನ್ನು ಡಾ॥ ರಣಜಿತ್‌ರವರು ಬಿಚ್ಚಿಡುತ್ತಾ ಸಾಗುತ್ತಾರೆ. ವೈದ್ಯಲೋಕವೇ ವಿಸ್ಮಯ ಪಡುವ ಹೃದಯದ ಕವಾಟಗಳನ್ನು ಬದಲಿಸುವ ಶಸ್ತ್ರಚಿಕಿತ್ಸೆ, ಅದಕ್ಕೆ ಪೂರಕವೆನಿಸುವ ಆಕಸ್ಮಿಕ ಸಂಶೋಧನೆಗಳು, ಕೃತಿಯ ಮೌಲ್ಯವನ್ನು ಹೆಚ್ಚಿಸಿವೆ. ಒಂದು ಕಾಲದಲ್ಲಿ ವೈದ್ಯ ವಿಜ್ಞಾನಕ್ಕೆ ಅಸಾಧ್ಯವಾದ, ಇಡೀ ವಿಶ್ವದ ಗಮನ ಸೆಳೆದ "ಕೃತಕ ಹೃದಯ" ಹಾಗೂ "ಹೃದಯ ಕಸಿ"ಯ ಕುರಿತಾದ ಸಂಶೋಧನೆ ಮತ್ತು ಸಾಧನೆಗಳು ಮಾನವನ ಅಪರಿಮಿತ ಬುದ್ಧಿಮತ್ತೆಗೆ ಸಾಕ್ಷಿಯೆನಿಸುವ ಅಪೂರ್ವ ಸಂಶೋಧನೆಗಳು. ಅದರ ಸಂಪೂರ್ಣ ವಿವರಗಳು ಈ ಕೃತಿಯಲ್ಲಿ ದಾಖಲಾಗಿರುವುದು ಈ ಕೃತಿಯ ವಿಶೇಷತೆಯಾಗಿದೆ. ಅದರೊಟ್ಟಿಗೆ ಹೃದ್ರೋಗ ಪತ್ತೆಮಾಡುವ ವಿಧಾನಗಳ ಸೃಷ್ಟಿ, ಶಸ್ತ್ರಕ್ರಿಯೆ ರಚಿತವಾಗಿ ಹೃದ್ರೋಗಗಳಿಗೆ ಚಿಕಿತ್ಸೆ ನೀಡುವ ವಿಧಾನಗಳ ಸೂಕ್ಷ್ಮ ವಿವರಣೆಗಳು ಓದುಗರನ್ನು ಆಕರ್ಷಿಸುತ್ತವೆ.

ಹೃದ್ರೋಗ ಚಿಕಿತ್ಸೆ–ಶಸ್ತ್ರಕ್ರಿಯೆಗಳ ಸಂಶೋಧನೆ ಕೇವಲ ಪಾಶ್ಚಿಮಾತ್ಯ

ರಾಷ್ಟ್ರಗಳಲ್ಲಿ ಮಾತ್ರ ಸಾಧ್ಯವಾಗಿದೆ ಎಂಬ ನಂಬಿಕೆಯನ್ನು ಹುಸಿಗೊಳಿಸಿ, ಭಾರತ ದಲ್ಲಿ ನಡೆದ ಹೃದಯ ಶಸ್ತ್ರಚಿಕಿತ್ಸೆಗಳ ಸಂಶೋಧನೆ ಮತ್ತು ಮೈಲುಗಲ್ಲುಗಳನ್ನು ಅತ್ಯಂತ ಪ್ರಾಮಾಣಿಕವಾಗಿ ದಾಖಲಿಸಿರುವ ಡಾ॥ ರಣಜಿತ್‌ರವರು ಹೇಗೆ ಭಾರತದಲ್ಲಿಂದು ವಿಶ್ವದರ್ಜೆಯ ಹೃದಯ ಚಿಕಿತ್ಸೆಯ ಪರಿಣಿತಿಯನ್ನು ಸಾಧಿಸಲಾಗಿದೆ ಎಂಬುದಾಗಿಯೂ ವಿವರಿಸುತ್ತಾರೆ. ಭಾರತದಲ್ಲಿ ಸಾಧನೆಗೈದ ಅನೇಕ ವೈದ್ಯರತ್ನರನ್ನು ಅತ್ಯಂತ ವಿನಮ್ರವಾಗಿ ಸ್ಮರಿಸಲಾಗಿದೆ. ವೈದ್ಯ ವಿಜ್ಞಾನಿ ಗಳಲ್ಲದೆ, ಹೃದಯ ಶಸ್ತ್ರಚಿಕಿತ್ಸೆ ಯಶಸ್ವಿಯಾಗಲು ರೂಪಿತವಾದ ಶಸ್ತ್ರಚಿಕಿತ್ಸಾ ಉಪಕರಣಗಳನ್ನು ಹಾಗೂ ಅವುಗಳನ್ನು ರೂಪಿಸಿದ ತಂತ್ರಜ್ಞರನ್ನು ಸ್ಮರಿಸಿದುದು ಈ ಕೃತಿಯ ಸಮಗ್ರತೆಗೆ ಕನ್ನಡಿಯಾಗಿದೆ.

ಹೃದಯ ರೋಗ ಚಿಕಿತ್ಸೆಯೂ ಸರ್ವರಿಗೂ ಲಭ್ಯವಾಗಬೇಕು ಹಾಗೂ ಅದು ಎಲ್ಲರಿಗೂ ಕೈಗೆಟಕುವ ದರದಲ್ಲಿ ಇರಬೇಕೆಂಬ ಅಭೀಪ್ಸೆಯನ್ನು ವ್ಯಕ್ತಪಡಿಸುವ ಲೇಖಕರು, ಭಾರತವೂ ಸೇರಿದಂತೆ ಇತರೆ ಕಡಿಮೆ ಮತ್ತು ಮಧ್ಯಮ ಆದಾಯದ ದೇಶಗಳು ಎದುರಿಸುತ್ತಿರುವ ಹೃದಯ ಶಸ್ತ್ರಚಿಕಿತ್ಸಕರ ಕೊರತೆಯನ್ನು ಎತ್ತಿ ತೋರಿಸುತ್ತಾ, ಮುಂದಿನ ಯುವ ವೈದ್ಯರಿಗೆ ಮನವಿಯನ್ನು ಮಾಡುತ್ತಾರೆ. ಇದು ಅವರ ಹೃದಯವಂತಿಕೆಗೆ ಸಾಕ್ಷಿಯಾಗಿದೆ.

ಒಟ್ಟಾರೆಯಾಗಿ, ಡಾ॥ ರಣಜಿತ್‌ರವರ ಈ "ಹೃದಯ ಸಂಗ್ರಾಮ" ಕೃತಿಯನ್ನು ಓದುವಷ್ಟರಲ್ಲಿ ನಮ್ಮಲ್ಲಿಯೆ ಒಂದು ಮಾನಸಿಕ ಸಂಗ್ರಾಮ ನಡೆದ ಅನುಭವವನ್ನು ತಂದುಕೊಡುತ್ತದೆ. ಪ್ರತಿ ಅಧ್ಯಾಯದ ಕೊನೆಯ ಭಾಗದಲ್ಲಿ ಆ ಅಧ್ಯಾಯದ ಘಟನೆಗಳ ಸೂಕ್ಷ್ಮ ಅವಲೋಕನ, ಅದರಿಂದ ವರ್ತಮಾನದ ಬದುಕಿಗೆ ಹೇಗೆ ಪ್ರೇರಣೆ ಪಡೆಯಬಹುದು ಎಂಬ ಲೇಖಕರ ವಿಮರ್ಶೆ ಅವರಲ್ಲಿಯ ಸೃಜನಶೀಲ ಬರವಣಿಗೆಗೆ ಸಾಕ್ಷಿಯಾಗಿದೆ. ಅವರ ಇಂತಹ ಪ್ರಯತ್ನ ಗಳಿಂದಲೇ, ಒಬ್ಬ ಭರವಸೆಯ ಕನ್ನಡ ವೈದ್ಯ ಬರಹಗಾರರಾಗಿ ರೂಪುಗೊಂಡಿ ದ್ದಾರೆ. ಅದಕ್ಕಾಗಿ ಅವರಿಗೆ ಹೃತ್ಪೂರ್ವಕ ಅಭಿನಂದನೆಗಳು.

ಪ್ರಸ್ತುತ ಕೃತಿಯು ಭಾರತೀಯ ವೈದ್ಯಕೀಯ ಸಂಘ ಕರ್ನಾಟಕ ರಾಜ್ಯ ಶಾಖೆಯ ಕನ್ನಡ ವೈದ್ಯ ಬರಹಗಾರರ ಸಮಿತಿಯ ವತಿಯಿಂದ ಕೆ.ಎಲ್.ಇ. ವಿಶ್ವವಿದ್ಯಾಲಯದ 'ಕನ್ನಡ ಬಳಗ', ಜವಾಹರಲಾಲ ನೆಹರು ವೈದ್ಯಕೀಯ ಮಹಾವಿದ್ಯಾಲಯ, ಬೆಳಗಾವಿ ಹಾಗೂ ಭಾರತೀಯ ವೈದ್ಯಕೀಯ ಸಂಘ, ಬೆಳಗಾವಿ ಶಾಖೆಗಳ ಸಂಯುಕ್ತ ಆಶ್ರಯದಲ್ಲಿ ನಡೆಯುತ್ತಿರುವ "ಕನ್ನಡ ವೈದ್ಯ ಬರಹಗಾರರ ಐದನೆಯ ರಾಜ್ಯ ಸಮ್ಮೇಳನ"ದಲ್ಲಿ ಲೋಕಾರ್ಪಣೆಯಾಗುತ್ತಿರು ವುದು ಅಭಿಮಾನದ ಸಂಗತಿಯಾಗಿದೆ. ಡಾ॥ ರಣಜಿತ್ ನಾಯಕರವರು

ಬೆಳಗಾವಿಯ ಕೆ.ಎಲ್.ಇ. ಡಾ. ಪ್ರಭಾಕರ ಕೋರೆ ಆಸ್ಪತ್ರೆ ಹಾಗೂ ವೈದ್ಯಕೀಯ ಸಂಶೋಧನಾ ಕೇಂದ್ರದಲ್ಲಿ ತಜ್ಞ ಹೃದಯ ಶಸ್ತ್ರಚಿಕಿತ್ಸಕರಾಗಿ ಕಾರ್ಯನಿರ್ವಹಿಸುತ್ತಿರುವುದು ಮತ್ತು ಇಂತಹವರು ಹೃದಯ ಚಿಕಿತ್ಸೆಯ ಮೈಲುಗಲ್ಲುಗಳ ಇತಿಹಾಸದ ಇಣುಕು ನೋಟವನ್ನು ನೀಡಿರುವುದು ಸಹಜವಾಗಿಯೇ ಕೃತಿಯ ದೃಢೀಕರಣವನ್ನು ಹೆಚ್ಚಿಸಿರುವುದು, ಸಂತೋಷದ ಸಂಗತಿಯಾಗಿದೆ.

ಪ್ರೀತಿಯ ಓದುಗರು, ಅದರಲ್ಲೂ ವೈದ್ಯ ವಿದ್ಯಾರ್ಥಿಗಳು ಈ ಕೃತಿಯನ್ನು ಅವಲೋಕಿಸಿ, ಅದರ ಮೌಲ್ಯಗಳನ್ನು ಅರಿಯುವ ಪ್ರಯತ್ನ ಮಾಡಿದರೆ ಕೃತಿಯ ಆಶಯಕ್ಕೊಂದು ಆಶ್ರಯ ಒದಗಿಸಿದಂತಾಗುತ್ತದೆ.

28–07–2024
ಬೆಳಗಾವಿ

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ಬೆಳಗಾವಿ

ಲೇಖಕರ ಮಾತು

ವೃತ್ತಿಯಲ್ಲಿ ಹೃದ್ರೋಗ ಶಸ್ತ್ರಕ್ರಿಯೆ ತಜ್ಞನಾಗಿರುವುದರಿಂದ ನಾನು ಕಳೆದ ಕೆಲ ವರ್ಷಗಳಲ್ಲಿ ಆರೋಗ್ಯ ಅದರಲ್ಲೂ ಪ್ರಮುಖವಾಗಿ ಹೃದಯ ರೋಗದ ಕುರಿತು ಜಾಗೃತಿ ಮೂಡಿಸಲು ಅಂಕಣ ಹಾಗೂ ಕೃತಿಯ ಮೂಲಕ ಪ್ರಯತ್ನಿಸಿದ್ದೇನೆ. ಹೃದಯ ಕುರಿತಾದ ವೈಜ್ಞಾನಿಕ ಅಂಕಿ–ಸಂಖ್ಯೆ, ಅದರ ರಚನೆ, ರೋಗ ಲಕ್ಷಣ, ಪತ್ತೆ, ಚಿಕಿತ್ಸೆ ಇವೆಲ್ಲದರ ಕುರಿತು ನಾನು ಈ ಹಿಂದೆ ಬರೆದಿದ್ದೇನೆ. ಈ ಕೃತಿಗಳಿಗೆ ದೊರೆತ ಜನ ಪ್ರತಿಕ್ರಿಯೆ, ಸ್ಪಂದನೆ, ಖುಷಿ ನೀಡಿದ್ದರೂ ಸಹ ಪ್ರತಿ ಬಾರಿಯೂ ನಾನು ಓದುಗರೊಂದಿಗೆ ಹಂಚಿಕೊಳ್ಳ ಬಯಸುವ ಹೃದಯದ ವಿಚಾರವೊಂದು ಹಾಗೇ ಉಳಿದಿದೆ ಎಂಬ ಭಾವನೆ ಸದಾ ಕಾಡುತ್ತಿತ್ತು. ವೈದ್ಯರಾಗಿ ನಾವು ಪ್ರತಿನಿತ್ಯವೂ ರೋಗಿಗಳಿಂದ ಕಲಿಯುತ್ತೇವೆ.

``He who studies medicine without books sails on uncharted sea, but he who studies medicines without patients doesn't go to sea at all."

–William Osler.

ವಿಲಿಯಂ ಓಸ್ಲರ್‌ರ ಈ ಮಾತಿನ ಅರ್ಥವೆಂದರೆ, ರೋಗಿಯನ್ನು ಅರಿಯದೇ ವೈದ್ಯಕೀಯ ಕಲಿಯುವುದು ಅಸಾಧ್ಯ. ಅಂತೆಯೇ ನನಗೂ ಕೂಡ ಓದುಗರಿಗೆ ರೋಗಿಗಳ ನೈಜ ಕಥೆಯನ್ನು ಪರಿಚಯಿಸದೇ ವೈದ್ಯಕೀಯ ಸಂಬಂಧ ಅರಿವು ಮೂಡಿಸುವುದು ಅಸಾಧ್ಯವೆನಿಸಿತು.

ನಾನು ಬರೆದ ಕೃತಿಗಳನ್ನು ಓದಿ ಅನೇಕ ರೋಗಿಗಳು ಒಳ್ಳೆಯ ಮಾಹಿತಿ ನೀಡಿದ್ದೀರಿ ಎಂದು ಹೇಳಿ ಶುಭ ಕೋರಿದ್ದಾರೆ. ಇವೆಲ್ಲದರ ನಡುವೆ ಅವರಲ್ಲಿ ಕೆಲ ಆತಂಕ–ಭವಿಷ್ಯದ ಚಿಂತೆ ಇರುವುದು ನನ್ನ ಅನುಭವಕ್ಕೆ ಬಂದಿದೆ. ಅದೆಷ್ಟೋ ಬಾರಿ ಈ ರೋಗಿಗಳ ಧೈರ್ಯ, ಇಚ್ಛಾಶಕ್ತಿ ನನಗೆ ಸ್ಫೂರ್ತಿಯಾಗಿರುತ್ತದೆ. ಆದರೆ, ಅವರನ್ನು ಹುರಿದುಂಬಿಸಲು ನಾನೇನು ಮಾಡಬಲ್ಲೆ ಎಂಬ ಪ್ರಶ್ನೆ ಮನದಲ್ಲಿ ಆಗಾಗ ಮೂಡುತ್ತದೆ, ಕಾಡುತ್ತದೆ. ಹಾಗಾಗಿ ವೈಜ್ಞಾನಿಕ ಮಾಹಿತಿಗೂ ಮಿಗಿಲಾಗಿ ರೋಗಿಗಳಿಗೆ ರೋಗ ಎದುರಿಸುವ ಮನೋಬಲ ನೀಡಬಲ್ಲ ಲೇಖನಗಳ ಅಗತ್ಯತೆ ನನ್ನನ್ನು ಕಾಡಿತು.

ಪ್ರತಿಯೊಬ್ಬ ರೋಗಿಯ ಸವಾಲು – ಕಥನ, ವಿಭಿನ್ನ ಹಾಗೂ ರೋಚಕ. ರೋಗ ಎದುರಿಸಿ ಗೆಲ್ಲುವವರು ಒಂದೆಡೆಯಾದರೆ, ಅದರೊಡನೆ ದಿಟ್ಟತನದಿಂದ ಹೋರಾಡಿ ಸ್ಫೂರ್ತಿಯಾಗಿ ಮನದಲ್ಲಿರುವವರು ಸಹ ಅನೇಕರಿರುತ್ತಾರೆ. ಹೃದ್ರೋಗಿಗಳ ಸಂಖ್ಯೆ ದಿನೇ ದಿನೇ ಹೆಚ್ಚುತ್ತಿದೆ. ಹೃದಯದ ಕುರಿತಾದ ನಮ್ಮೆಲ್ಲರ ಕುತೂಹಲವೂ ಸಹ ಅಧಿಕಗೊಳ್ಳುತ್ತಿದೆ. ಇಂದಲ್ಲ ನಾಳೆ ನಾವೆಲ್ಲರೂ ಹೃದ್ರೋಗವನ್ನು ನೇರವಾಗಿ ಇಲ್ಲವೇ ಪರೋಕ್ಷವಾಗಿ ಎದುರಿಸಲೇಬೇಕು.

ನಮ್ಮ "ಹೃದಯ" ಇಂದು ರಣಾಂಗಣವಾಗಿದೆ. ಈ ಹೃದಯದಲ್ಲಿ ನಿರಂತರವಾಗಿ "ಸಂಗ್ರಾಮ" ಅಥವಾ "ಯುದ್ಧ" ನಡೆಯುತ್ತಲೇ ಇದೆ. ಹೃದಯ ರೋಗದ ಪತ್ತೆಯಲ್ಲಿ ಅಥವಾ ಅದರ ಚಿಕಿತ್ಸಾ ಕ್ರಮಗಳನ್ನು ಮಾನವ ಕಂಡುಕೊಂಡ ರೀತಿ ಎಲ್ಲವೂ ರೋಚಕ ಕಥನಗಳಾಗಿವೆ. ನನ್ನ ಅನುಭವ ದೊಂದಿಗೆ ಮನಕ್ಕೆ ಬಂದ ವೈಜ್ಞಾನಿಕ ಸಂಗತಿಯನ್ನು ಒಟ್ಟೊಟ್ಟಿಗೆ ವಿವರಿಸಿ ರೋಗಿಗಳನ್ನು ಪ್ರೇರೇಪಿಸುವ ಉದ್ದೇಶವನ್ನು ಈ ಮೂಲಕ ಹೊಂದಿದ್ದೇನೆ. ರೋಗಿಗಳ ಗೌಪ್ಯತೆ ಹಾಗೂ ಖಾಸಗಿತನಕ್ಕೆ ಧಕ್ಕೆಯಾಗಬಾರದೆಂಬ ಕಾರಣಕ್ಕೆ ಹೆಸರು, ಊರು ಇತ್ಯಾದಿಯನ್ನು ಹಂಚಿಕೊಳ್ಳುವುದು ನನ್ನ ವೃತ್ತಿಯಲ್ಲಿ ಅಸಾಧ್ಯ. ಆದರೆ, ಅವರ ವೀರಗಾಥೆಗಳನ್ನು ಅನಾಮಿಕವಾಗಿಯಾದರೂ ಕೊಂಡಾಡಿ ಹಲವರನ್ನು ಪ್ರೇರೇಪಿಸಬೇಕು ಎಂಬ ತುಡಿತ ನನ್ನದಾಗಿದೆ.

ನನ್ನ ಬರವಣಿಗೆಗೆ ಪ್ರೇರಕ ಶಕ್ತಿ ನನ್ನ ತಂದೆ. ಬದುಕಿನಲ್ಲಿ ತಪ್ಪು ತಿದ್ದುವ ಅಮ್ಮ, ಲೇಖನಗಳ ಪ್ರೂಫ್ ರೀಡರ್ ಸಹ ಆಗಿದ್ದಾರೆ. ನನ್ನೆಲ್ಲ ಪ್ರಯತ್ನಗಳಿಗೆ ಆಧಾರ ಸ್ತಂಭ ಹಾಗೂ ಸ್ಫೂರ್ತಿ ನೀಡುವವರೆಂದರೆ ನನ್ನ ತಂಗಿ ರಮ್ಯ, ಸಮೃದ್ಧಿ, ಪುಟ್ಟ ಅಭಿಮನ್ಯು ಹಾಗೂ ಅರ್ಜುನಣ್ಣ. ನನ್ನ ಬರವಣಿಗೆಯನ್ನು ಬೆಳೆಸಿದ ನನ್ನೆಲ್ಲ ಗುರುಗಳು ಮತ್ತು ಕರಾವಳಿ ಮುಂಜಾವು ಬಳಗವನ್ನು ಸದಾ ನೆನೆಯುತ್ತೇನೆ. ಪುಸ್ತಕ ಪ್ರಕಟಿಸುವ ಮೂಲಕ ನನ್ನನ್ನು ಪೋಷಿಸುತ್ತಿರುವ 'ಸಪ್ನ ಬುಕ್ ಹೌಸ್'ಗೆ ನಾನು ಚಿರಋಣಿಯಾಗಿದ್ದೇನೆ. ಕನ್ನಡ ಸೇವೆಯನ್ನು ಮುಂದು ವರೆಸಲು ಸದಾ ಪ್ರೋತ್ಸಾಹ ನೀಡುತ್ತಿರುವ ಹಿರಿಯರಾದ ಆರ್. ದೊಡ್ಡೆಗೌಡ ಸರ್ ಹಾಗೂ ನಿತಿನ್ ಷಾ ಸರ್ ಅವರಿಗೆ ಕೃತಜ್ಞನಾಗಿದ್ದೇನೆ.

ಹೃದಯ ಶಸ್ತ್ರಕ್ರಿಯೆಯ ಕುರಿತು ಮೂಲಭೂತ ಪಾಠ ಕಲಿಸಿದ ನನ್ನ ಗುರುಗಳಾದ ಡಾ॥ ಚಂದ್ರಪ್ರಕಾಶ ಶ್ರೀವಾತ್ಸವ ಅವರಿಗೆ ಈ ಪುಸ್ತಕವನ್ನು ಅರ್ಪಿಸಿ ದ್ದೇನೆ. ತಮ್ಮ ಅನಾರೋಗ್ಯದ ನಡುವೆಯೂ ಇತರರ ಚಿಕಿತ್ಸೆಯಲ್ಲಿ ತೊಡಗಿರುವ ಅವರು ಓರ್ವ ಮಾದರಿ ವ್ಯಕ್ತಿಯಾಗಿದ್ದಾರೆ. ಅಂತೆಯೇ ನನಗೆ ಮಾರ್ಗದರ್ಶನ ಮಾಡುವ ಎಲ್ಲ ಹಿರಿಯ–ಕಿರಿಯ ಸಹದ್ಯೋಗಿಗಳನ್ನು ನಾನು ಸ್ಮರಿಸುತ್ತೇನೆ.

ನನ್ನೆಲ್ಲ ಪುಸ್ತಕಗಳಿಗೆ ತುಂಬಾ ಅಚ್ಚುಕಟ್ಟಾಗಿ ಅಕ್ಷರ ಜೋಡಣೆ ಮಾಡುವ ರೇಣುಕಾ, ಮಂಗಳಾ, ದಿವ್ಯ ಪ್ರಿಂಟ್ರಾನಿಕ್ಸ್ ತಂಡ, ಮುಖಪುಟ ವಿನ್ಯಾಸ ಮಾಡುವ ಶ್ರೀಪಾದರವರಿಗೆ ನಾನು ಧನ್ಯವಾದಗಳನ್ನು ಅರ್ಪಿಸುತ್ತೇನೆ. ಪುಸ್ತಕ ಮುದ್ರಣದಲ್ಲಿ ಬೆಂಬಲವಾಗಿರುವ ಪೂರ್ಣಿಮಾ ಪ್ರಿಂಟರ್ಸ್ ತಂಡದವರಿಗೆ ಕೃತಜ್ಞನಾಗಿದ್ದೇನೆ.

ಈ ಪುಸ್ತಕದ ಕರಡು ಪ್ರತಿ ಓದಿ, ಮುನ್ನುಡಿ ಬರೆದು ಹಾರೈಸಿದ ಡಾ. ಅವಿನಾಶ ಕವಿ ಅವರಿಗೆ ನಾನು ಋಣಿಯಾಗಿದ್ದೇನೆ.

ಪ್ರತಿನಿತ್ಯ ನಾವು ಹಲವಾರು ರೋಗಿಗಳೊಂದಿಗೆ ಸಮಾಲೋಚನೆ ನಡೆಸುತ್ತೇವೆ. ಅವರಿಂದ ಅನೇಕ ಜೀವನ ಮೌಲ್ಯವನ್ನು ಕಲಿಯುತ್ತೇವೆ. ರೋಗದ ಕುರಿತು ತಿಳಿಯುತ್ತೇವೆ. ಅವರಿಂದಲೇ ನಾವು ಬೆಳೆಯುತ್ತೇವೆ. ನಾನು ಸದಾ ನನ್ನೆಲ್ಲ ಹೃದ್ರೋಗಿ ಬಂಧುಗಳಿಗೆ ಕೃತಜ್ಞನಾಗಿದ್ದೇನೆ. ಅವರ ಸೇವೆಗಾಗಿ ಅವಕಾಶ ನೀಡಿದಕ್ಕಾಗಿ ಅವರೆಲ್ಲರಿಗೆ ಋಣಿಯಾಗಿದ್ದೇನೆ.

"ಹೃದಯ ಸಂಗ್ರಾಮ" ಎಂಬ ಈ ಕೃತಿ ಎದೆಗುಂದದ ಹೃದಯಗಳ ಸಾಹಸಗಾಥೆಯಾಗಿಸಲು ಪ್ರಯತ್ನಿಸಿದ್ದೇನೆ. ಇದರಲ್ಲಿ ಹೃದಯದ ಕುರಿತು ಹಿರಿಯ ವಿಜ್ಞಾನಿಗಳು ಪಟ್ಟ ಪ್ರಯಾಸ, ರೋಗಿಗಳ ನಿಜ ಅನುಭವ, ಹೃದ್ರೋಗ ಗೆದ್ದು ನಮ್ಮ ನಡುವೆ ಬದುಕುತ್ತಿರುವ ಸಾಮಾನ್ಯರಲ್ಲಿನ ಅಸಾಮಾನ್ಯರ ಕೆಲ ಕಥೆಗಳನ್ನು ಉಲ್ಲೇಖಿಸಲು ಪ್ರಯತ್ನಿಸಿದ್ದೇನೆ. ಇದು ಸಂಪೂರ್ಣ ವೈಜ್ಞಾನಿಕ ಕೃತಿಯಲ್ಲ. ಇದು ಕಥೆ–ಕಾದಂಬರಿಯೂ ಅಲ್ಲ. ಇದು ನಾನು ಕಲಿತ ಪಾಠ, ಅರ್ಥೈಸಿಕೊಳ್ಳಲು ಪ್ರಯತ್ನಿಸಿದ ರೀತಿ, ಹೃದಯದ ಕುರಿತ ಕಲಿಕೆಯಲ್ಲಿ ಉಂಟಾದ ಅನುಭವ ಸೇರಿದಂತೆ ಇನ್ನೊಬ್ಬರಿಗೆ ಮುಖ್ಯವಾಗಿ ಹೃದ್ರೋಗಿಗಳಿಗೆ ರೋಗ ಮೆಟ್ಟಿ ನಿಲ್ಲಲು ಅನುವು ಮಾಡಿಕೊಡಬೇಕೆಂಬ ಹಂಬಲದ ಮಿಶ್ರಣವಾಗಿದೆ. ಈ ನನ್ನ ಪ್ರಯತ್ನಕ್ಕೆ ತಮ್ಮೆಲ್ಲರ ಆಶೀರ್ವಾದವಿರಲಿ.

ಡಾ॥ ರಣಜಿತ್ ಬೀರಣ್ಣ ನಾಯ್ಕ ಕೆಂಚನ್,
ಹಿರೇಗುತ್ತಿ

ಪರಿವಿಡಿ

1)	ಅಸಾಧ್ಯವ ಮೀರಿದ ಅಸಂಪ್ರದಾಯಿಕ ನಡೆ	00
2)	ಮಹಾಯುದ್ಧದ ನಡುವೆ ಹೃದಯ ಸ್ಪರ್ಶಿಸಿದ ಯೋಧರು	00
3)	ಗುರುವಿನ ಅನುಪಸ್ಥಿತಿಯಲ್ಲಿ ದಿಟ್ಟ ಹೆಜ್ಜೆ	00
4)	"ನೀಲಿ ಮಕ್ಕಳ" ಪಾಲಿಗೆ ವರವಾದ ತ್ರಿಮೂರ್ತಿಗಳ ಸಂಗಮ	00
5)	ವಿಶ್ವದಲ್ಲೇ ಪ್ರಥಮ ಹಾಗೂ ಕೊನೆಯದಾಗಿ ತನ್ನ ಹೃದಯದ ಸೆಲ್ಫಿ ಕ್ಲಿಕ್ಕಿಸಿದ ಹತಮಾರಿ	00
6)	ಸೋಲೊಪ್ಪದ ನಿರಂತರ ಪ್ರಯತ್ನದ ಫಲ–ಕೃತಕ ಹೃದಯ ಶ್ವಾಸಕೋಶ ಯಂತ್ರ	00
7)	ಹೃದಯದ ರಾಣಿ–ಸಿಸಿಲಿಯಾ ಬೊವೆಲಿಕ್	
8)	ಜೀವ ಉಳಿಸಲು ಶೇ. 200 ರಷ್ಟು ಅಪಾಯ (Risk) ಎದುರಿಸಿದ ಧೀರ	00
9)	ಹೃದಯದ ಬಾಗಿಲು ಅಥವಾ ಕವಾಟ ಬದಲಿಸಿದ ಧೀರರು	00
10)	ಹೃದಯ ಶಸ್ತ್ರಕ್ರಿಯೆಗೆ ಪೂರಕವೆನಿಸಿದ ಆಕಸ್ಮಿಕ ಸಂಶೋಧನೆಗಳು	00
11)	ವಿಶ್ವದ ಗಮನ ತನ್ನತ್ತ ಸೆಳೆದ ಹೃದಯದ ಕಸಿ	00
12)	ವೃತ್ತಿಪರ ಪೈಪೋಟಿಗೆ ಕಾರಣವಾದ ಕದ್ದ "ಕೃತಕ ಹೃದಯ"	00
13)	ಆಕಸ್ಮಿಕವಾಗಿ ಸೆರೆಸಿಕ್ಕ ಕಿರೀಟ ಧಮನಿಗಳು	00
14)	ಹೃದಯದ ಕೊರೆನರಿ ಬೈಪಾಸ್ ಜನಪ್ರಿಯಗೊಳಿಸಿದ ದುರಂತ "ನಾಯಕ"	00
15)	ಬಲೂನಿನಿಂದ ರಕ್ತ ಹರಿವಿನ ಅಡೆ–ತಡೆ ನಿವಾರಿಸಿದ ಮಾಂತ್ರಿಕರು	00
16)	ಜೀವ ಉಳಿಸುವ "ಸ್ಟೆಂಟ್" ಹೆಸರಿನ ಮೂಲ	00
17)	ಚಿಕ್ಕದು ಚೊಕ್ಕ ಹಾಗೂ ಸುಂದರ	00
18)	ತೂರುನಳಿಕೆ ಆಧಾರಿತ ಕವಾಟ ಬದಲಾವಣೆ	00
19)	ಪ್ರಾಣಿಗಳಿಂದ ಮನುಜನಿಗೆ ಜೀವದಾನ	00
20)	ಭಾರತದಲ್ಲಿ ಹೃದಯಗಳನ್ನು ಸರಿಪಡಿಸಿದ ಮೊದಲಿಗರು	00
21)	ಹೃದ್ರೋಗ ಚಿಕಿತ್ಸೆಯ ಚಿತ್ರಣ ಬದಲಿಸಿದ 20ನೇ ಶತಮಾನದ ಸಂಶೋಧನೆಗಳು	00

22) "ಮೇಡ್ ಇನ್ ಇಂಡಿಯಾ" ಹೃದಯ ಕವಾಟ 00
23) ಜೀವಕ್ಕೆ ಬೆಲೆ ಕಟ್ಟಲು–ಕಟ್ಟದಿರಲು ಪ್ರಯತ್ನಿಸುತ್ತಿರುವ ವೈದ್ಯರು 00
24) ಹೃದಯ ಶಸ್ತ್ರಕ್ರಿಯೆಗೆ ಬೇಕಾದ ಉಪಕರಣಗಳ ಕೆಲ ವಿನ್ಯಾಸಕರ ನೆನೆಯುತ್ತ 00
25) ಸುದೀರ್ಘ ಹೃದಯ ಸಂಗ್ರಾಮ ಇನ್ನೂ ಮುಂದುವರೆಯಲಿದೆ 00

1

ಅಸಾಧ್ಯವ ಮೀರಿದ ಅಸಂಪ್ರದಾಯಿಕ ನಡೆ

ಹೃದಯದ ಶಸ್ತ್ರಕ್ರಿಯೆ ಇಂದು ಜಗತ್ತಿನಾದ್ಯಂತ ವೇಗ ಪಡೆದು ಕೊಳ್ಳುತ್ತಿದ್ದು, ಸಾಮಾನ್ಯವೆನಿಸಿಕೊಳ್ಳುತ್ತಿದೆ. ವಿಜ್ಞಾನ ಕಲಿತವರೆಲ್ಲ ಅಂಧತ್ವ ಹೊಂದಿರಲಾರರು ಎಂಬುದು ನಮ್ಮ ಸಾಮಾನ್ಯ ಭಾವನೆ. ವಿಜ್ಞಾನಿಗಳು – ವೈದ್ಯರುಗಳು ಸಹ ತಮ್ಮದೇ ಆದ ತತ್ವ – ಸಿದ್ಧಾಂತಗಳ ಕುರಿತು ಅಂಧ ಭಕ್ತಿ – ಅಭಿಮಾನ ಹೊಂದಿರುವ ಸಾಧ್ಯತೆ ಇರುತ್ತದೆ. 19ನೇ ಶತಮಾನದಲ್ಲಿ ಇದೇ ರೀತಿಯ ಅಂಧತ್ವ ಹೃದಯದ ಕುರಿತು ಜನಮಾನಸದಲ್ಲಿ ಬೇರೂರಿತ್ತು.

ಹೋಮರ್‌ನ "ಇಲಿಯಡ್", "ಒಡಿಸ್ಸಿ" ಸೇರಿದಂತೆ ಭಾರತೀಯ ಮಹಾಕಾವ್ಯಗಳಾದ "ರಾಮಾಯಣ", ಮಹಾಭಾರತದ ಕಾಲದಿಂದಲೂ ನಮಗೆ ಇರಿತದಿಂದಾಗುವ ಗಾಯಗಳ ವರ್ಣನೆ ತಿಳಿದಿದೆ. ಆ ದಿನಗಳಲ್ಲಿ ಭರ್ಜಿ, ಈಟಿ ಯಂತಹ ಚೂಪಾದ ಆಯುಧಗಳಿಂದ ಯುದ್ಧಗಳು ನಡೆಯುತ್ತಿದ್ದು, ಇರಿತದಿಂದ ಸಾವುಂಟಾಗುತ್ತಿತ್ತು ಎಂಬುದು ನಮಗೆ ತಿಳಿದ ವಿಚಾರ. ಹೃದಯಕ್ಕೆ ಉಂಟಾಗುವ ಇರಿತದಿಂದ ಸಾವು ನಿಶ್ಚಿತ ಎಂಬುದು ಇಂದಿಗೂ ನಮಗೆ ತಿಳಿದಿರುವ ವಿಚಾರವಾಗಿದೆ. ಎರಡನೇ ಶತಮಾನದಲ್ಲಿ ಗ್ಯಾಲನ್, 3ನೇ ಶತಮಾನದಲ್ಲಿ ಅರಿಸ್ಟಾಟಲ್, 16ನೇ ಶತಮಾನದಲ್ಲಿ ಆಂಬ್ರೋಸ್ ಪಾರೆ ಮುಂತಾದವರು ಹೃದಯದ ಗಾಯಗಳ ಕುರಿತು ವಿವಿಧವಾಗಿ ವಿವರಿಸಿದ್ದು ಇದೆ.

ಹೃದಯವೆನ್ನುವುದು ಒಂದು ಮಾಂಸದ ಉಂಡೆಯಿದ್ದಂತೆ. ಅದಕ್ಕೆ ಸ್ವಲ್ಪ ತರಚು ಗಾಯವಾದರೂ ಸಾಕು, ರಕ್ತ ಚಿಮ್ಮುತ್ತದೆ. ಹೀಗೆ ಚಿಮ್ಮಿದ ರಕ್ತ ಹೃದಯದ ಸುತ್ತ ಶೇಖರಣೆಗೊಳ್ಳುತ್ತದೆ. ಹೀಗೆ ಕೆಲವೊಮ್ಮೆ ರಕ್ತವು ಹೃದಯ ಹಾಗೂ ಅದರ ಹೊರಪದರವಾದ ಪೆರಿಕಾರ್ಡಿಯಮ್‌ನ ನಡುವೆ ಶೇಖರಣೆ ಗೊಳ್ಳುತ್ತದೆ. ಇದನ್ನು ಹೊರತೆಗೆಯದಿದ್ದಲ್ಲಿ ರಕ್ತವು ಹೃದಯದ ಮೇಲೆ ಒತ್ತಡ ಹಾಕಿ ಹೃದಯದ ಕಾರ್ಯಕ್ಷಮತೆಗೆ ಅಡೆ–ತಡೆಯಾಗಿ ಸಾವು ಸಂಭವಿಸಬಹುದು.

ಕೆಲವೊಮ್ಮೆ ಟಿ.ಬಿ., ಕ್ಯಾನ್ಸರ್ ಇನ್ನಿತರೆ ಕಾಯಿಲೆಯಿಂದಾಗಿಯೂ ಸಹ ನೀರಿನಂಶವೂ ಹೃದಯದ ಸುತ್ತ ಶೇಖರಣೆಯಾಗಬಹುದು. ಅದನ್ನು ನಾವು ಪೆರಿಕಾರ್ಡಿಯಲ್ ಎಫ್ಯೂಶನ್ ಅನ್ನುತ್ತೇವೆ. ಈ ಬಗೆಯಲ್ಲಿ ಶೇಖರಣೆಯಾದ ನೀರಿನಂಶವನ್ನು ಶಸ್ತ್ರಚಿಕಿತ್ಸೆಯ ಮೂಲಕ ಹೊರತೆಗೆದ ಖ್ಯಾತಿ ಫ್ರಾನ್ಸಿಸ್ಕೊ ರೊಮೆರವರದ್ದಾಗಿದೆ. 1801ರಲ್ಲಿ ಗ್ರಾಂಡ್ ಆರ್ಮಿಯ ಮುಖ್ಯ ಶಸ್ತ್ರಚಿಕಿತ್ಸಕರಾದ ಬ್ಯಾರನ್ ಲ್ಯಾರಿಯವರು ಎದೆಯ ನಡುಮೂಳೆಯಾದ ವಕ್ಷಾಸ್ಥಿ(Sternum)ಯ ಕೆಳಭಾಗದಲ್ಲಿ ಸಣ್ಣ ರಂಧ್ರ ಮಾಡಿ ಹೃದಯದ ಹೊರಪದರದಿಂದ ನೀರಿನಂಶ ಹೊರತೆಗೆಯಬಹುದೆಂದು ತೋರಿಸಿಕೊಟ್ಟರು. ಹಾಗಾಗಿ ದೇಹದ ಕ್ಸಿಫಾಯಿಡ್‌ನ ಕೆಳಭಾಗವನ್ನು ಇಂದಿಗೂ ಲ್ಯಾರಿ ಪಾಯಿಂಟ್ ಎಂದು ಗುರುತಿಸುತ್ತಾರೆ. ಅವರು ಆತ್ಮಹತ್ಯೆಗೆ ಪ್ರಯತ್ನಿಸಿ ಹೃದಯಕ್ಕೆ ಗಾಯ ಮಾಡಿಕೊಂಡಿದ್ದ ಸೈನಿಕನ ಪ್ರಾಣ ಉಳಿಸಲು ಈ ಪ್ರಯತ್ನವನ್ನು ಮಾಡಿದರು. ಆದರೆ, ಹೃದಯವನ್ನು ಮುಟ್ಟುವ, ಅದಕ್ಕೆ ಹೊಲಿಗೆ ಹಾಕುವ ಸಾಹಸ ಮಾಡಲಿಲ್ಲ.

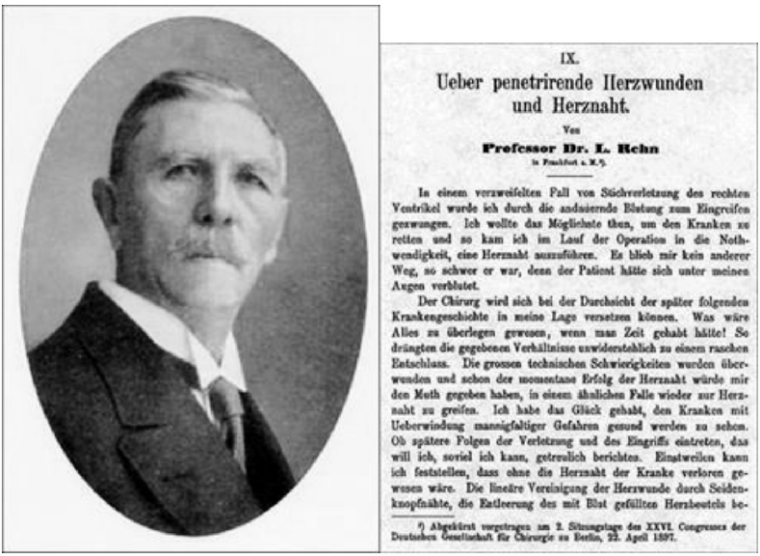
1882ರಲ್ಲಿ ಬ್ಲಾಕ್ ಹಾಗೂ ಇನ್ನಿತರೆ ತಜ್ಞರು ಹೃದಯ ಶಸ್ತ್ರಕ್ರಿಯೆಯ ಕುರಿತು ಪ್ರಾಣಿಗಳಲ್ಲಿ ಪ್ರಯತ್ನಿಸುತ್ತಿದ್ದರೂ ಸಹ ಆಗಿನ ಖ್ಯಾತ ತಜ್ಞರನೇಕರು ಈ ಕುರಿತು ಮೂಗು ಮುರಿಯಲಾರಂಭಿಸಿದರು. 1883ರಲ್ಲಿ ಆಸ್ಟ್ರಿಯಾದ ಪ್ರಖ್ಯಾತ ಶಸ್ತ್ರಕ್ರಿಯಾ ತಜ್ಞರು ಹಾಗೂ ಇಂದಿಗೂ ವೈದ್ಯರು ಆರಾಧಿಸುವ ಥಿಯೋಡಾರ್ ಬಿಲ್ರೋತ್ ಹೃದಯವನ್ನು ಹೊಲಿಯಲು ಪ್ರಯತ್ನಿಸುವ ವಿಜ್ಞಾನಿ, ವೈದ್ಯರು ತನ್ನ ಸಂಗಡಿಗರ ಗೌರವ ಕಳೆದುಕೊಳ್ಳಬೇಕಾದೀತು ಎಂದು ಘೋಷಿಸಿ ಎಚ್ಚರಿಸಿದ್ದರು.

ಈ ಬಗೆಯ ನಿರಾಶಾವಾದ, "ಅಸಾಧ್ಯ" ಎಂಬ ನಂಬಿಕೆಯ ನಡುವೆ ಮೊದಲ ಬಾರಿಗೆ ಹೃದಯದ ಮಧ್ಯದ ಪದರವಾದ ಮಯೋಕಾರ್ಡಿಯಮ್‌ನ ಗಾಯವನ್ನು ಹೊಲಿಯಲು ಪ್ರಯತ್ನಿಸಿದವರು ನಾರ್ವೆಯ ಆಕ್ಸೆಲ್ ಕ್ಯಾಪ್ಲೆನ್, 1895ರಲ್ಲಿ 24 ವರ್ಷದ ಹುಡುಗನ ಎದೆ ಭಾಗಕ್ಕೆ ಆದ ಇರಿತವನ್ನು ಹಾಗೂ ಹೃದಯದ ಎಡ ಹೃತ್ಕುಕ್ಷಿಯಲ್ಲಾದ 2 ಸೆಂಟಿಮೀಟರ್ ಉದ್ದದ ಗಾಯವನ್ನು ಹೊಲಿಯಲು ಅವರು ಪ್ರಯತ್ನಿಸಿದರು. ಆದರೆ ಅವರು ಸಫಲರಾಗಲಿಲ್ಲ.

ಇದಾದ ಕೆಲವೇ ತಿಂಗಳುಗಳಲ್ಲಿ ಅಂದರೆ 1896ರ ಸಪ್ಟೆಂಬರ್ 9ರಂದು ಜರ್ಮನಿಯ ಫ್ರಾಂಕ್‌ಫರ್ಟ್‌ನಲ್ಲಿ ಲುಡ್ವಿಗ್ ರೆಹ್ನ ಎಂಬ ವೈದ್ಯ, ರೋಗಿಯ ನರಳಾಟ ನೋಡಲಾರದೇ ಆತನನ್ನು ಬದುಕಿಸಬೇಕೆಂಬ ಹಠದಿಂದ "ಅಸಂಪ್ರದಾಯಿಕ" ನಡೆ ಇಡಲು ಸಜ್ಜಾದ. ವಿಲ್ಹೆಮ್ ಜಸ್ಟಸ್ ಎಂಬ 22 ವರ್ಷದ ಮಾಲಿ ಅಥವಾ ತೋಟಗಾರನಿಗೆ ಚೂರಿಯ ಇರಿತವಾಗಿತ್ತು. ಸಪ್ಟೆಂಬರ್ 7ರಂದು ಆತ ಆಸ್ಪತ್ರೆಗೆ ದಾಖಲಾಗಿದ್ದ. ಅಂದಿನ ಕಾಲದಲ್ಲಿ ಲಭ್ಯವಿದ್ದ

ಚಿಕಿತ್ಸೆಯಂತೆ ಆತನ ಆರೈಕೆ ಮುಂದುವರೆದಿತ್ತು. ಆದರೆ, ರಕ್ತಸ್ರಾವ ನಿಲ್ಲಲಿಲ್ಲ. ರಕ್ತವು ಎದೆ ಹಾಗೂ ಶ್ವಾಸಕೋಶದ ಸುತ್ತ ಶೇಖರಣೆಯಾಗಲಾರಂಭಿಸಿತ್ತು. ರೋಗಿಯ ಉಸಿರಾಟ ತೀವ್ರವಾಗಿತ್ತು. ರಜೆ ಮೇಲಿದ್ದ ಲುಡ್ವಿಗ್ ಆಗ ತಾನೇ ಹಿಂದಿರುಗಿದ್ದ. ಈ ರೋಗಿಯ ನರಳಾಟ ಆತನಿಂದ ಸಹಿಸಲಾಗಲಿಲ್ಲ. ರೋಗಿಗೆ ಎದೆಯ ಎಡಭಾಗದಲ್ಲಿ ಸುಮಾರು 1.5 ಸೆಂ.ಮೀ. ಗಾಯವಿರುವುದನ್ನು ಆತ ಗಮನಿಸಿದ.

ರೋಗಿ ಕುಸಿಯುತ್ತಾನೆ ಎಂದು ಖಾತರಿಯಾಗುತ್ತಿದ್ದಂತೆ ಆತ ರೋಗಿಯ ಎದೆಯ ಎಡಭಾಗದಲ್ಲಿ 14 ಸೆಂ.ಮೀ. ಛೇದ ಮಾಡಿದ. ರೋಗಿಯ ಎಡಭಾಗದ ಐದನೆ ಪಕ್ಕೆಲುಬನ್ನು ತುಂಡರಿಸಿ ಹೃದಯದ ಹೊರ ಪದರವನ್ನು ತಲುಪಿದ. ಹೊರಪದರವನ್ನು ಕತ್ತರಿಸುತ್ತಿದ್ದಂತೆ ಹಿಂದೆಂದೂ ಜಗತ್ತು ಕಂಡಿರದ ನೋಟಕ್ಕೆ ಸಾಕ್ಷಿಯಾದ. ವೇಗವಾಗಿ ಬಡದುಕೊಳ್ಳುತ್ತಿದ್ದ ಹೃದಯ ಹಾಗೂ ಅದರಿಂದ ಚಿಮ್ಮುತ್ತಿದ್ದ ಗಾಢ ಬಣ್ಣದ ರಕ್ತವನ್ನು ಗಮನಿಸಿದ. ಹೃದಯದಲ್ಲಿ ಆಗಿದ್ದ ಗಾಯವನ್ನು ಆತ ಚಿಮ್ಮುಟದಿಂದ ಗಟ್ಟಿಯಾಗಿ ಹಿಡಿದ. ಬಲಭಾಗದ ಹೃತ್ಕುಕ್ಷಿಯಿಂದ ರಕ್ತಸ್ರಾವ ಆಗುತ್ತಿತ್ತು. ಆತ ಗಾಯದ ಎಡಭಾಗದಿಂದ ಸಿಲ್ಕ್ ಹೊಲಿಗೆಯಿಂದ ಹೊಲಿಯಲು ಆರಂಭಿಸಿದ. ಆತ ಮೂರನೇ ಹೊಲಿಗೆ ತೆಗೆದುಕೊಳ್ಳುತ್ತಿದ್ದಂತೆ ರಕ್ತಸ್ರಾವ ನಿಂತಿತು.



IX.

Ueber penetrirende Herzwunden und Herznaht.

Von

Professor Dr. L. Rehn

in Frankfurt a. M.[1]

In einem verzweifelten Fall von Stichverletzung des rechten Ventrikel wurde ich durch die andauernde Blutung zum Eingreifen gezwungen. Ich wollte das Möglichste thun, um den Kranken zu retten und so kam ich im Lauf der Operation in die Nothwendigkeit, eine Herznaht auszuführen. Es blieb mir kein anderer Weg, so schwer er war, denn der Patient hätte sich unter meinen Augen verblutet.

Der Chirurg wird sich bei der Durchsicht der später folgenden Krankengeschichte in meine Lage versetzen können. Was wäre Alles zu überlegen gewesen, wenn man Zeit gehabt hätte! So drängten die gegebenen Verhältnisse unwiderstehlich zu einem raschen Entschluss. Die grossen technischen Schwierigkeiten wurden überwunden und schon der momentane Erfolg der Herznaht würde mir den Muth gegeben haben, in einem ähnlichen Falle wieder zur Herznaht zu greifen. Ich habe das Glück gehabt, den Kranken mit Ueberwindung mannigfaltiger Gefahren gesund werden zu sehen. Ob spätere Folgen der Verletzung und des Eingriffs eintreten, das will ich, soviel ich kann, getreulich berichten. Einstweilen kann ich feststellen, dass ohne die Herznaht der Kranke verloren gewesen wäre. Die lineäre Vereinigung der Herzwunde durch Seidenknopfnähte, die Entleerung des mit Blut gefüllten Herzbeutels be-

[1]) Abgekürzt vorgetragen am 2. Sitzungstage des XXVI. Congresses der Deutschen Gesellschaft für Chirurgie zu Berlin, 22. April 1897.

ಲುಡ್ವಿಗ್ ರೆಹ್ನ ಹಾಗೂ ಆತನ ಅನುಭವದ ಕುರಿತು ಆತನ ಬರಹ.

ಎದೆ ಭಾಗದಲ್ಲಿ ಶೇಖರಣೆಯಾಗಿದ್ದ ರಕ್ತದ ಹೆಪ್ಪು ತೆಗೆದು ಚೆನ್ನಾಗಿ ತೊಳೆದು ಮುರಿದಿದ್ದ ಮೂಳೆಯನ್ನು ಮರುಜೋಡಿಸಿ ಎದೆ ಭಾಗದ ಗಾಯವನ್ನು ಹೊಲಿದು ಹೊರಬಂದ ಲುಡ್ವಿಗ್ ಇತಿಹಾಸ ಸೃಷ್ಟಿಸಿದ್ದ.

ಪ್ರಪ್ರಥಮ ಬಾರಿಗೆ ರೆಹ್ನ ಹೃದಯದ ಗಾಯವನ್ನು ಯಶಸ್ವಿಯಾಗಿ ಹೊಲಿದಿದ್ದ. ಅದರ ಆರು ತಿಂಗಳ ತರುವಾಯ ಆತ ಈ ಕುರಿತು ಜರ್ಮನ್ ಶಸ್ತ್ರಕ್ರಿಯೆ ಸೊಸೈಟಿಯಲ್ಲಿ ತನ್ನ ಈ ರೋಮಾಂಚಕ ಅನುಭವವನ್ನು ಹಂಚಿ ಕೊಂಡಿದ್ದ. ಈ ಅನುಭವದ ತರುವಾಯ 1907ರೊಳಗೆ ಆತ 124ಕ್ಕೂ ಅನೇಕ ಪ್ರಕರಣಗಳಲ್ಲಿ ಹೃದಯದ ಗಾಯವನ್ನು ಶೇ. 60ರಷ್ಟು ಶ್ರೇಯದೊಂದಿಗೆ ಹೊಲಿದ. ಹೃದಯಕ್ಕೆ ಗಾಯವಾದರೂ ರೋಗಿ ಬದುಕಬಲ್ಲ ಎಂಬುದನ್ನು ತೋರಿಸಿಕೊಟ್ಟ. ಹೃದಯ ಶಸ್ತ್ರಕ್ರಿಯೆಗೆ ಸಾಂಪ್ರದಾಯಿಕ ನಂಬಿಕೆಗಳನ್ನು ಗಾಳಿಗೆ ತೂರಿ ಅಸಾಧ್ಯವ ಮೀರಿ ಅಡಿಪಾಯ ಹಾಕಿದ. ಸ್ಫೂರ್ತಿಯಾದ. ಹೃದಯ ಶಸ್ತ್ರಕ್ರಿಯೆ ಆರಂಭವಾಗಿದ್ದೇ ಇಂತಹ ಬಗೆಯ ದಿಟ್ಟತನದಿಂದ ಎಂದರೆ ತಪ್ಪಾಗಲಾರದು.

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ಸುದೀರ್ಘ ಹೃದಯ ಸಂಗ್ರಾಮ ಇನ್ನೂ ಮುಂದುವರೆಯಲಿದೆ

ಹೃದಯಕ್ಕೂ ಹಾಗೂ ನಮ್ಮ ದೇಹದ ಚರ್ಮದ ನಡುವಿನ ಅಂತರ 6–7 ಸೆಂಟಿಮೀಟರ್ ಇರಬಹುದು. ಆದರೆ ಈ ದೂರವನ್ನು ಕ್ರಮಿಸಿ ಶಸ್ತ್ರಕ್ರಿಯೆ ಮಾಡಲು ಶಕ್ತರೆನಿಸಲು ನಮಗೆ ಹಲವಾರು ಶತಮಾನಗಳೇ ಬೇಕಾದವು. ಕಳೆದ 125 ವರ್ಷಗಳಲ್ಲಿ ಹೃದಯ ಶಸ್ತ್ರಕ್ರಿಯೆ ಊಹೆಗೂ ಮೀರಿದ ಪ್ರಗತಿ ಕಂಡಿದೆ. ಇವುಗಳಿಗೆ ಕಾರಣರಾದ ಅನೇಕ ಘಟನೆ–ಮಹನೀಯರನ್ನು ನಾವು ಈಗಾಗಲೇ ನೆನೆದಿದ್ದೇವೆ. ಆದರೆ "ಹೃದಯ–ಸಂಗ್ರಾಮ" – ಪ್ರತಿಯೋರ್ವರಿಗೂ ಸೂಕ್ತ ಕಾಲದಲ್ಲಿ ಸರಿಯಾದ ಹೃದಯ ಚಿಕಿತ್ಸೆ ಒದಗಿಸುವ ಹೋರಾಟ ಇನ್ನೂ ಪೂರ್ತಿಯಾಗಿಲ್ಲ. ಈ ಸುದೀರ್ಘವಾದ ಪ್ರಯತ್ನ ಇನ್ನೂ ಅನೇಕ ದಶಕಗಳವರೆಗಾದರೂ ಮುಂದುವರೆಯಲಿದೆ.

ವಿಶ್ವಾದ್ಯಂತ ಇಂದಿಗೂ 6 ಬಿಲಿಯನ್‌ಗೂ ಅಧಿಕ ಮಂದಿಗೆ ಸೂಕ್ತ, ಸುರಕ್ಷಿತ, ಸಮಯೋಚಿತ ಹಾಗೂ ಕೈಗೆಟಕುವ ದರದಲ್ಲಿ ಹೃದಯ ಶಸ್ತ್ರಕ್ರಿಯೆ ಸೇವೆ ಲಭ್ಯವಿಲ್ಲ ಎಂದು ಅಂದಾಜಿಸಲಾಗಿದೆ. ಇಂದು ಹೃದ್ರೋಗ ವಿಶ್ವಾದ್ಯಂತ ಅತೀ ಹೆಚ್ಚು ಸಾವು–ನೋವಿಗೆ ಕಾರಣವೆನಿಸಿದೆ. ಅವುಗಳಲ್ಲಿ ಕನಿಷ್ಟವೆಂದರೂ ಮೂರನೇ ಒಂದು ಭಾಗದಷ್ಟು ಜನರಿಗಾದರೂ ಹೃದಯ ಶಸ್ತ್ರಕ್ರಿಯೆ ಅಗತ್ಯವೆನ್ನಬಹುದು. ಹೃದಯ ಕಿರೀಟ ಧಮನಿಯ ರೋಗದೊಂದಿಗೆ 200 ಮಿಲಿಯನ್‌ಗೂ ಅಧಿಕ ಮಂದಿ ವಾಸಿಸುತ್ತಿದ್ದಾರೆ. 2050 ರ ವೇಳೆಗೆ 2 ಬಿಲಿಯನ್‌ಗೂ ಅಧಿಕ ಮಕ್ಕಳು ಜನ್ಮಜಾತವಾಗಿ ಬಂದ ಹೃದ್ರೋಗದಿಂದ ಬಳಲಲಿದ್ದಾರೆ ಎಂದು ಅಂದಾಜಿಸಲಾಗಿದೆ. ಬಡವರನ್ನು ಮುಖ್ಯವಾಗಿ ಕಾಡುವ ರ್ಯುಮಾಟಿಕ್ ಹೃದ್ರೋಗ ಜಗತ್ತಿನಾದ್ಯಂತ 30–40 ಮಿಲಿಯನ್ ಜನರನ್ನು ಕಾಡುತ್ತಿದೆ ಎಂದು ನಂಬಲಾಗಿದೆ.

ಹೆಚ್ಚು ಆದಾಯವುಳ್ಳ ರಾಷ್ಟ್ರದಲ್ಲಿ ಪ್ರತಿ 10 ಲಕ್ಷ ಜನರಿಗೆ 7 ರಿಂದ 8

ಹೃದಯ ಶಸ್ತ್ರಕ್ರಿಯ ತಜ್ಞರಿದ್ದರೆ ಬಡರಾಷ್ಟ್ರಗಳಲ್ಲಿ ಒಬ್ಬರೂ ಇಲ್ಲ ಎನ್ನುವುದು ದುರ್ದೈವ. ಹೃದಯ ಶಸ್ತ್ರಕ್ರಿಯೆ ಸೇವೆಗಳಲ್ಲಿ ಪ್ರಾದೇಶಿಕ ವ್ಯತ್ಯಾಸಗಳೂ ಸಹ ಅಧಿಕವಾಗಿದೆ. ಉದಾಹರಣೆಗೆ ಭಾರತದ ನಕಾಶೆಯಲ್ಲಿ ಮುಂಬಯಿ ಹಾಗೂ ವಿಶಾಖಪಟ್ಟಣಂನ ಜೋಡಿಸುವಂತೆ ನಾವು ಗೆರೆ ಎಳೆದರೆ, ಅದರ ಉತ್ತರಕ್ಕೆ ಇರುವ ಪ್ರದೇಶಗಳಲ್ಲಿ ಹೃದಯ ಕಸಿಯ ಸಂಖ್ಯೆ ವಿರಳ. ಆದರೆ ದೇಶದ ದಕ್ಷಿಣ ಭಾಗದಲ್ಲಿ ಹೃದಯ ಶಸ್ತ್ರಕ್ರಿಯೆಯ ಸೇವೆ ವಿಪುಲವಾಗಿ ಬೆಳೆದಿದೆ ಎಂದು ಹೆಮ್ಮೆ ಪಡಬಹುದು. ಈ ಬೆಳವಣಿಗೆಯ ಹೊರತಾಗಿಯೂ ಸಹ ದಕ್ಷಿಣ ಭಾರತದ ಅನೇಕ ಜಿಲ್ಲೆಗಳಿಗೆ ಸಮಯಕ್ಕೆ ಸರಿಯಾಗಿ ಚಿಕಿತ್ಸೆ ಒದಗಿಸಬಲ್ಲ ಹೃದಯ ಸಂಬಂಧಿ ಆಸ್ಪತ್ರೆ, ಆರೋಗ್ಯ ಸೇವೆಗಳಿಲ್ಲ.

ಹೃದಯ ರೋಗ ಚಿಕಿತ್ಸೆ ಎಲ್ಲರಿಗೂ ಸೂಕ್ತ ಸಮಯದಲ್ಲಿ ದೊರೆಯ ಬೇಕಾದರೆ ಹೃದಯ ಶಸ್ತ್ರಕ್ರಿಯ ತಜ್ಞರ ಸಂಖ್ಯೆ ಅಧಿಕವಾಗಬಹುದು. ಹಾಗಾಗಿ ಹೆಚ್ಚೆಚ್ಚು ವೈದ್ಯಕೀಯ ವಿದ್ಯಾರ್ಥಿಗಳು ಈ ವಿಭಾಗದತ್ತ ಆಸಕ್ತಿ ಬೆಳೆಸಿಕೊಳ್ಳುವಂತೆ ಅವರನ್ನು ಪ್ರೇರೇಪಿಸಬೇಕು. ಹೃದಯ ಶಸ್ತ್ರಕ್ರಿಯೆ ವಿಭಾಗದಲ್ಲಿ ಕೆಲಸ ಹೆಚ್ಚು, ಆದಾಯ ಕಡಿಮೆ ಎಂಬ ಕಾರಣಕ್ಕೆ ಅನೇಕ ಯುವ ವೈದ್ಯರು ಇದನ್ನು ಆಯ್ದುಕೊಳ್ಳಲು ಹಿಂದೇಟು ಹಾಕುತ್ತಿದ್ದಾರೆ. ಹೃದಯ ಶಸ್ತ್ರಕ್ರಿಯೆ ಬೆಳೆದು ಬಂದ ರೀತಿ, ಅನೇಕಾನೇಕ ವೈದ್ಯರುಗಳ ಅವಿರತ ಪ್ರಯತ್ನದ ಕುರಿತು ಯುವ ಮನಗಳ ಗಮನ ಸೆಳೆಯಲೆಂದು ಈ ಕೃತಿ ರಚಿಸಿದ್ದೇನೆ.

ಹೃದಯ ಶಸ್ತ್ರಕ್ರಿಯೆ ಅಗತ್ಯವಿರುವ ರೋಗಿಗೆ ಸಕಾಲಕ್ಕೆ ದೊರೆಯಬೇಕೆಂಬ ಇಚ್ಛೆ ಒಂದೆಡೆಯಾದರೆ ಈ ದಿಶೆಯಲ್ಲಿ ಜನಸೇವೆಯಲ್ಲಿ ತೊಡಗಿರುವ ಹೃದಯ ಶಸ್ತ್ರಕ್ರಿಯೆ ತಜ್ಞರ ಶ್ರಮಕ್ಕೆ ಸೂಕ್ತವಾದ ಗೌರವ–ಆದಾಯ ದೊರಕಿದಾಗ ಮಾತ್ರ ಈ ಸಂಗ್ರಾಮವನ್ನು ಮುಂದುವರೆಸಬಹುದೆಂಬ ಆಶಯ ಮನದಲ್ಲಿ ಮೂಡಿದೆ. ಹೃದಯ ಶಸ್ತ್ರಕ್ರಿಯೆ ನಿಂತ ನೀರಲ್ಲ. ಹಾಗಾಗಿ ಈ ವಿಷಯಕ್ಕೆ ಸಂಬಂಧಿಸಿದಂತೆ ಸಂಶೋಧನೆಗಳು ನಿರಂತರವಾಗಿ ನಡೆಯುತ್ತಿರಲಿ. ಸೂಕ್ತವಾದ ಪ್ರೋತ್ಸಾಹ–ಧನ ಸಹಾಯವನ್ನು ಸರ್ಕಾರ–ಸಮಾಜ ಮಾಡಿದಾಗ ಮಾತ್ರ ಪ್ರಗತಿ ಸಾಧ್ಯ. ಹಿರಿಯರು ಆರಂಭಿಸಿದ ಈ "ಹೃದಯ ಸಂಗ್ರಾಮ"ದಲ್ಲಿ ಪೀಡಿತ ಎಲ್ಲ ಹೃದಯಗಳ ವೇದನೆಗೆ ಉತ್ತರ ಸಿಕ್ಕು, ಪ್ರತಿಯೋರ್ವರ ಹೃದಯವೂ ಸಮೃದ್ಧಿ ಯನ್ನು ಹೊಂದಲಿ ಎಂದು ದೇವರಲ್ಲಿ ಪ್ರಾರ್ಥಿಸುತ್ತೇನೆ.

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ಹೃದಯ ಸಂಗ್ರಾಮ

ಡಾ॥ ರಣಜಿತ್ ನಾಯಕರವರು ನಮ್ಮ ದಿನಮಾನದ ಯುವ ವೈದ್ಯ ಲೇಖಕರಾಗಿ, ಕನ್ನಡ ವೈದ್ಯ ಸಾಹಿತ್ಯಕ್ಕೆ ತಮ್ಮ ಅಮೂಲ್ಯ ಸೇವೆಯನ್ನು ಸಲ್ಲಿಸುತ್ತಿದ್ದಾರೆ. ಚಿಕ್ಕ ವಯಸ್ಸಿನಲ್ಲಿಯ ಸಂಸ್ಕಾರ, ಅವರ ಕುಟುಂಬದ ಪ್ರೋತ್ಸಾಹ, ಶಿಕ್ಷಕರ ಮಾರ್ಗದರ್ಶನ, ತಾನು ಕಲಿತ ವಿದ್ಯೆಯನ್ನು ಜನಸಾಮಾನ್ಯರಿಗೂ ತಲುಪಿಸಬೇಕೆಂಬ ಹಂಬಲಗಳು ಮೇಳೈಸಿ, ತಮ್ಮ ನಿರಂತರ ಸವಾಲುಗಳ ವೃತ್ತಿಯಾದ ಹೃದಯ ಶಸ್ತ್ರಚಿಕಿತ್ಸೆಯೊಂದಿಗೆ ಸಾಹಿತ್ಯದ ಅಭಿರುಚಿಯನ್ನು ಜಾಗೃತವಾಗಿಟ್ಟು, ಆ ನಿಟ್ಟಿನಲ್ಲಿ ಪ್ರಯತ್ನಪೂರ್ವಕವಾಗಿ ಮುತುವರ್ಜಿಯಿಂದ ಶ್ರಮವಹಿಸಿ ಈಗಾಗಲೇ ಆರು ಮೌಲಿಕ ಕೃತಿಗಳನ್ನು ರಚಿಸಿರುವ ಇವರು ನಮ್ಮ ಯುವ ವೈದ್ಯರಿಗೆ ಮಾದರಿಯಾಗಿದ್ದಾರೆ. 'ಹೃದಯ ಸಂಗ್ರಾಮ' ಅವರ ಏಳನೇಯ ಕೃತಿಯಾಗಿದೆ. ಸ್ವತಃ ಹೃದ್ರೋಗ ಚಿಕಿತ್ಸೆಯ ಪರಿಣಿತರಾಗಿ ಹೃದಯ ಸಂಬಂಧಿ ರೋಗಗಳ ಚಿಕಿತ್ಸೆ, ಅದನ್ನು ತಡೆಗಟ್ಟುವ ವಿಧಾನಗಳ ಕುರಿತಾಗಿ ಜನಸಾಮಾನ್ಯರನ್ನು ದೃಷ್ಟಿಯಲ್ಲಿರಿಸಿಕೊಂಡು ಕನ್ನಡದಲ್ಲಿ ಅನೇಕ ಅಂಕಣಗಳನ್ನು ಹಾಗೂ ಪುಸ್ತಕಗಳನ್ನು ರಚಿಸಿರುವ ಇವರು ಪ್ರಸ್ತುತ ಕೃತಿಯಲ್ಲಿ ಹೃದ್ರೋಗ ಚಿಕಿತ್ಸೆ ಹಾಗೂ ಶಸ್ತ್ರಕ್ರಿಯೆಗಳ ಆವಿಷ್ಕಾರ, ಸಂಶೋಧನೆಗಳ ಮೈಲುಗಲ್ಲುಗಳನ್ನು, ಅದರ ಸಾಹಸಗಾಥೆಗಳನ್ನು ಬಿಚ್ಚಿಡುವ ಪ್ರಯತ್ನವನ್ನು ಸಮರ್ಥವಾಗಿ ಮಾಡಿದ್ದಾರೆ.

ಡಾ॥ ರಣಜಿತ್ ಅವರೇ ಹೇಳಿಕೊಂಡಂತೆ, 'ಹೃದಯ ಸಂಗ್ರಾಮ' ಎಂಬ ಕೃತಿಯಲ್ಲಿ ಹೃದ್ರೋಗಕ್ಕೆ ಶಾಶ್ವತ ಪರಿಹಾರ ಕೈಗೊಳ್ಳಬೇಕೆಂಬ ದಿಟ್ಟ ಹೃದಯಗಳ ಸಾಹಸಗಾಥೆಯನ್ನು ಕಟ್ಟಿಕೊಟ್ಟು, ಹೃದಯ ಚಿಕಿತ್ಸಕರು-ಶಸ್ತ್ರಚಿಕಿತ್ಸಕರು ಪಟ್ಟ ಪ್ರಯಾಸ, ಅವರೊಂದಿಗೆ ಸಹಕರಿಸಿದ ವ್ಯಕ್ತಿಗಳ, ಚಿಕಿತ್ಸೆಗೆ ಒಪ್ಪಿದ ರೋಗಿಗಳ, ಕೆಲವೊಮ್ಮೆ ಚಿಕಿತ್ಸೆ ಫಲಿಸದೆ ಪ್ರಾಣತೆತ್ತವರ ಕಥೆಗಳ ಸಾರವೇ ಅಡಗಿದೆ.

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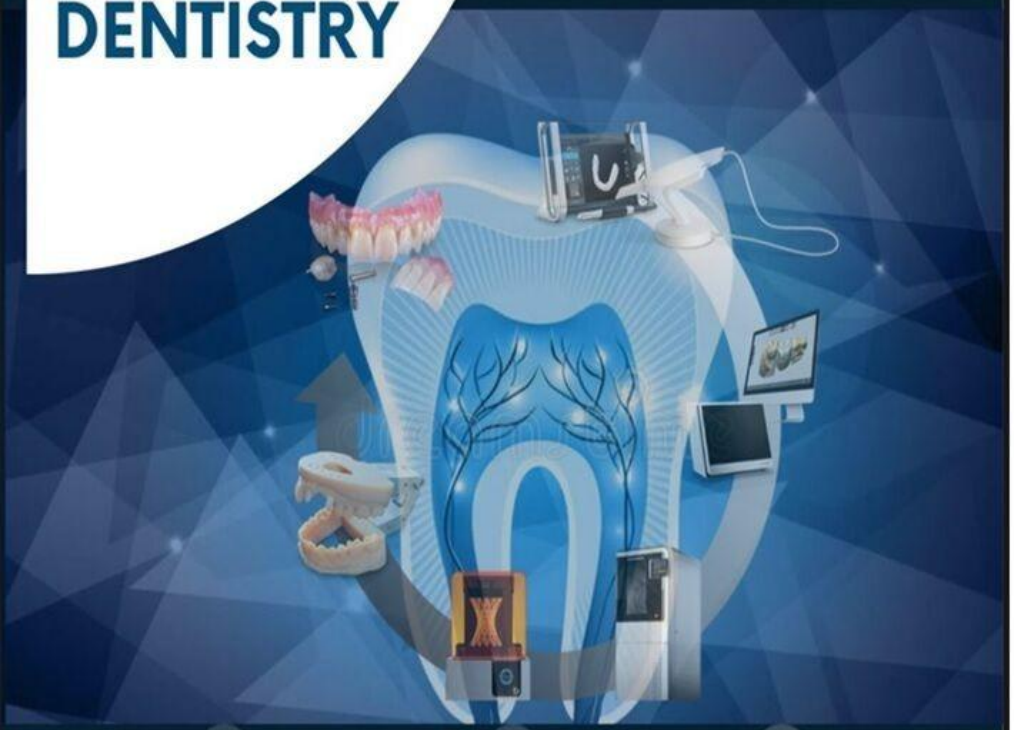

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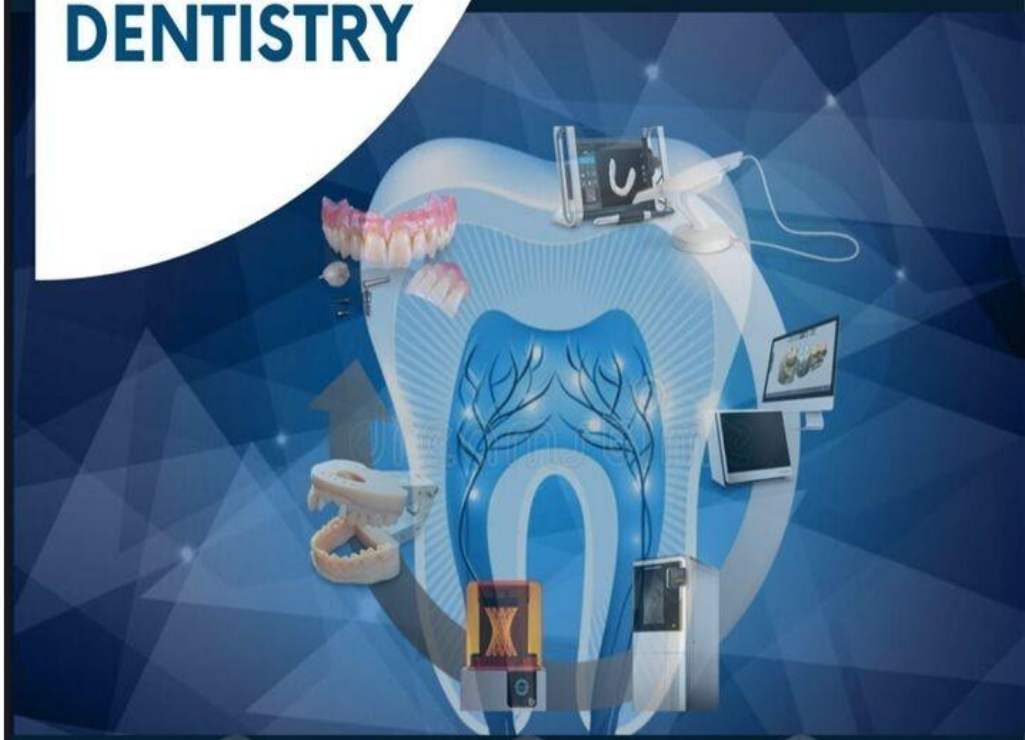
FUTURE TRENDS IN DIGITAL DENTISTRY

FIRST EDITION

FUTURE TRENDS IN DIGITAL DENTISTRY

EXPLORING THE LATEST INNOVATIONS IN DENTAL TECHNOLOGY



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Future Trends In Digital Dentistry

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Dr. Vinodhini V is an Endodontist and Aesthetic Dentist with four years of experience. She works as an Assistant professor (Department of conservative Dentistry and Endodontics) in Mahatma Gandhi Postgraduate Institute of Dental sciences, Puducherry. She completed Bachelor of Dental surgery from Rajah Muthiah Dental College and Hospital, Annamalai University, Chidambaram. Then masters of dental surgery in conservative dentistry and Endodontics from government dental college (Kozhikode) with work experience of one year as senior resident in government dental college, Thrissur. Through guidance from experienced professionals, she learnt & practice conservative Dentistry & Endodontist as an art, much passionately.

Dr. Radhika Thakkar

Dr. Radhika Thakkar's journey through the realm of dentistry spans over a decade, showcasing her evolution from a dedicated student to a passionate educator. Her educational foundation began with a Bachelor of Dental Surgery (BDS) from Narsinhbhai Patel Dental College & Hospital, Hemchandracharya North Gujarat University, India, where she completed a 4-year dental school program followed by a 1-year internship in 2012. Building upon this foundation, she pursued post-graduate diplomas in Medico Legal System (PGDMLS) and Hospital Management (PGDHM) from Symbiosis International University, Pune, India in 2013, highlighting her drive for a holistic understanding of the field. She spent transformative years in India as private practice owner before pursuing a Masters of Public Health (MPH) from St. John's University, NY, USA in 2018. Further enriching her expertise in the dental health ecosystem.

Her commitment to a comprehensive approach is evident in her subsequent Research

Fellowship at the Eastman Institute for Oral Health, University of Rochester, NY, USA, a two-year Advanced Education in General Dentistry Residency (AEGD), and a Graduate Medical Education Residency (GME) Program at the same institute, completed between 2021 and 2024. Dr. Thakkar's profound commitment to evidence-based dentistry is evident through her extensive research portfolio of 20 internationally recognized scientific papers. Her pursuit of continuous learning saw her participation in the DentalXP NYU Online Implant Fellowship Program, demonstrating her commitment to keeping up with the latest advancements in the field. Dr. Thakkar's professional trajectory includes impressive accolades such as the title of Fellow from ICOI (International Congress of Oral Implantologists). An advocate of aesthetic dentistry, Dr. Thakkar earned the title of Fellow from the Institute of Laser and Aesthetic Medicine and Dentistry, affiliated with the University of Greifswald, Germany. She has also presented her findings at prestigious conferences like American Academy of Implant Dentistry (AAID) and American Academy of Developmental Medicine & Dentistry (AADMD) and Academy of General Dentistry (AGD).

Outside of her professional endeavors, Dr. Thakkar's zest for exploration takes the form of outdoor adventures encompassing camping, biking, kayaking, and extensive travel. She has even taken up the challenge of learning the piano and basic Spanish, showcasing her multifaceted approach to life. With an unwavering dedication to both her profession and her

personal growth, Dr. Radhika Thakkar continues to make a profound impact on the field of dentistry while embracing a rich and fulfilling life beyond it.

Dr. Mayank Sharma

Dr. Mayank Sharma practices Advanced General and Cosmetic Dentistry in California, USA. He has close to 15 years of clinical experience spanning across India, Australia, and the United States. He graduated with Honors from the University of California at San Francisco and is a Fellow with the Academy of General Dentistry, an extremely prestigious achievement which has been awarded to only 6% to General Dentists in the US and Canada. He's a Spear dentist and member of multiple international and national dental organizations like American Dental Association, International Team of Implantologists, American Academy of Clear Aligners, International Association of Dental Traumatology, etc. With multiple publications including co-authoring a book on facial trauma under his belt, Dr. Sharma is also on the Editorial board of an internationally acclaimed dental journal and is a peer reviewer in multiple journals. He believes in decreasing the environmental impact and promoting patient wellness by supporting green dental practices through his work. He is an enthusiastic volunteer and has supported The American Red Cross, The Indian Red Cross

Society, Mission of Mercy project, and other local community clinics. Dr. Sharma is described by his patients as a compassionate and a highly skilled dentist who is known for great chairside manners and excellency in managing patients with chronic dental anxiety. He is an avid musician and a guitarist who also loves to travel and read. Dr. Sharma believes that "All the hard work put into training as a dentist is paid off after seeing my patient's beautiful smile!"

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DEDICATION

This Book is dedicated to

My family and teachers

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Chapter 1: Introduction to Digital Dentistry

Evolution of Digital Dentistry

The evolution of digital dentistry stands as a testament to the remarkable fusion of technology with the art and science of oral healthcare. Over the past few decades, the field of dentistry has undergone a profound transformation, driven by advancements in digital technologies that have reshaped every facet of patient care, practice management, and research. The journey of digital dentistry's evolution is a captivating narrative that showcases how innovation, precision, and patient-centricity have become integral to modern dental practice.

Early Foundations and Technological Shifts

The seeds of digital dentistry were sown in the late 20th century when computers and imaging technologies began to infiltrate dental practices. The introduction of intraoral cameras and digital radiography marked the initial steps toward digitizing patient records and diagnostic processes. These early foundations laid the groundwork for the paradigm-shifting changes that would follow.

Innovation Takes Center Stage

The turn of the millennium witnessed an explosion of innovation in digital dentistry. Computer-Aided Design (CAD) and Computer-Aided Manufacturing (CAM) technologies emerged as game-changers, enabling the creation of precise dental restorations. The digital workflow evolved further with the integration of 3D printing, making it possible to fabricate complex dental structures with unparalleled accuracy.

Rise of Virtual Treatment Planning

Virtual treatment planning revolutionized dental procedures, especially in the fields of orthodontics and implantology. Advanced imaging techniques like Cone Beam Computed Tomography (CBCT) combined with software solutions allowed practitioners to meticulously plan implant placements, orthodontic treatments, and complex surgical procedures. This shift not only enhanced predictability but also improved patient outcomes.

Patient-Centered Care in a Digital Age

One of the most significant impacts of digital dentistry has been the empowerment of patients in their own oral health journeys. From visualizing treatment outcomes through simulations to participating in shared decision-making, patients have become active collaborators in their care. The advent of patient education apps, virtual consultations, and wearable devices has elevated patient engagement to unprecedented levels.

Beyond Restorations: Tissue Regeneration and Bioprinting

Digital dentistry's evolution extended beyond traditional restorative procedures. Tissue engineering and bioprinting introduced the possibility of regenerating dental tissues like enamel, dentin, and even entire teeth. This revolutionary approach holds promise for restoring oral function and aesthetics in ways previously unimaginable.

Ethical and Regulatory Considerations

As the digital landscape expanded, so did the need for ethical and regulatory frameworks. Data security, patient privacy, and ethical use of emerging technologies became paramount. The dental community collaborated with regulatory bodies to ensure that digital dentistry's potential was harnessed responsibly and safely.

The Future: A Nexus of Innovation and Patient-Centricity

As we stand on the precipice of the future, digital dentistry continues to evolve. The convergence of AI, blockchain, telehealth, and more promises to create an interconnected ecosystem that further enhances patient care, enables more precise diagnostics, and shapes personalized treatment approaches. The evolution of digital dentistry is a journey that epitomizes the boundless potential of human ingenuity, and its unfolding narrative holds the promise of rewriting the future of oral healthcare for generations to come.

Emergence of Digital Dentistry

The emergence of digital dentistry marks a transformative turning point in the field of oral healthcare, ushering in an era defined by technological innovation, precision, and patient-centered care. As traditional dental practices have evolved to incorporate digital technologies, the landscape of dentistry has been reshaped, offering practitioners and patients unprecedented opportunities for enhanced diagnostics, treatment planning, and therapeutic outcomes. This chapter delves into the captivating story of how digital dentistry emerged as a powerful force, redefining the way dental professionals approach their craft and empowering individuals to actively engage in their oral health journeys.

Origins in Technological Advancements

The roots of digital dentistry trace back to the convergence of computing power, imaging technologies, and dental science. Early applications, such as digital radiography and intraoral cameras, laid the foundation for the digitization of patient records and diagnostic processes. These initial steps paved the way for a monumental shift that would forever alter the landscape of dentistry.

Transformation through Computer-Aided Design (CAD) and Manufacturing (CAM)

The emergence of digital dentistry gained momentum with the rise of Computer-Aided Design (CAD) and Computer-Aided Manufacturing (CAM) technologies. These innovations marked a departure from traditional manual techniques, enabling dental professionals to design and fabricate restorations with unparalleled precision. The integration of CAD/CAM systems streamlined workflows, allowing for quicker turnaround times and minimizing human error.

Digital Imaging and Diagnostic Precision

Digital dentistry's emergence revolutionized diagnostics through advanced imaging techniques like Cone Beam Computed Tomography (CBCT) and digital impressions. These technologies provided three-dimensional insights into patients' oral anatomy, enabling practitioners to visualize pathologies, plan treatments, and simulate outcomes with a level of accuracy previously unattainable.

Empowering Patients through Visualization

One of the defining aspects of the emergence of digital dentistry is its impact on patient engagement. Interactive treatment simulations and visualizations allow patients to understand their conditions and proposed interventions more comprehensively. Empowered with

knowledge, patients actively participate in decision-making and treatment planning, forging a collaborative partnership with dental professionals.

Virtual Treatment Planning and Beyond

Digital dentistry's evolution extended to encompass virtual treatment planning, especially in complex procedures like orthodontics and implantology. Practitioners harnessed digital tools to meticulously plan treatments, simulate outcomes, and optimize surgical approaches. This shift not only enhanced the accuracy of procedures but also contributed to better patient experiences and outcomes.

Ethical Considerations and Regulatory Adaptations

As the digital landscape expanded, ethical considerations and regulatory frameworks came to the forefront. Data privacy, patient consent, and responsible technology use became focal points of discussion. Dental professionals collaborated with regulatory bodies to establish guidelines that ensure patient safety and uphold ethical standards in the digital realm.

The Promise of an Evolving Future

The emergence of digital dentistry is an ongoing journey that continues to unfold with remarkable potential. As artificial intelligence, telehealth, bioprinting, and more become integral to oral healthcare, the landscape is poised to undergo further transformation. The

promise of an evolving future lies in the fusion of technology with clinical expertise, aiming to provide patients with personalized, precise, and accessible oral healthcare like neverbefore.

In conclusion, the emergence of digital dentistry is a chapter in the story of healthcare evolution that highlights the remarkable synergy between technology and the art of dentistry. From improved diagnostics to patient empowerment and ethical considerations, digital dentistry's ascent signifies a new era of precision, collaboration, and patient-centered care that holds immense promise for the future of oral healthcare.

Diagnostic Advancements

Diagnostic advancements have emerged as a cornerstone of modern healthcare, and dentistry is no exception. In the realm of oral healthcare, diagnostic innovations have transformed the way dental professionals assess, identify, and address dental conditions and concerns. This chapter explores the remarkable journey of diagnostic advancements in dentistry, highlighting how cutting-edge technologies and techniques have ushered in an era of precision, early detection, and tailored patient care.

Evolution from Traditional to Digital Diagnostics

The evolution of diagnostic methods in dentistry has seen a shift from traditional analog techniques to digital precision. Traditional X-rays and physical impressions have given way to digital radiography, cone beam computed tomography (CBCT), and intraoral scanners. These digital tools provide high-resolution images that offer unparalleled insights into dental and craniofacial structures.

3D Imaging and Visualization

Three-dimensional imaging has revolutionized diagnostic capabilities in dentistry. CBCT scans offer a comprehensive view of oral and maxillofacial structures, aiding in the accurate diagnosis of conditions ranging from impacted teeth to complex craniofacial anomalies. This level of detail enhances treatment planning, surgical precision, and patient communication.

Digital Impressions and Virtual Models

Digital impressions have transformed the process of capturing oral anatomy. Intraoral scanners create accurate digital models of patients' teeth and soft tissues, eliminating the discomfort of traditional impressions. These digital models facilitate treatment planning, orthodontic assessments, and the design of restorations with unmatched precision.

AI-Powered Diagnostics and Predictive Analytics

The emergence of artificial intelligence (AI) has elevated diagnostic capabilities to new heights. AI algorithms can analyze radiographic images, identifying subtle patterns and anomalies that may not be evident to the naked eye. Additionally, AI-powered predictive analytics aid in risk assessment and early detection of oral diseases, enabling proactive intervention.

Oral Cancer Detection and Screening

Diagnostic advancements have significantly improved the detection of oral cancer. Innovations like fluorescence imaging and advanced spectroscopy techniques enable early identification of potentially malignant lesions, enhancing the chances of successful treatment and improved patient outcomes.

Precision in Treatment Planning

Diagnostic advancements have redefined treatment planning, enabling dental professionals to create personalized treatment plans that align with each patient's unique oral anatomy. This precision is particularly evident in orthodontics and implantology, where virtual treatment planning based on digital models ensures optimal outcomes.

Patient-Centered Care and Communication

Modern diagnostic tools empower patients to actively participate in their care. Visualizations of treatment plans and diagnostic findings foster clearer communication between dental professionals and patients, enabling shared decision-making and ensuring that patients are informed partners in their oral health journey.

Future Horizons and Ongoing Evolution

The trajectory of diagnostic advancements in dentistry shows no signs of slowing down. As technology continues to evolve, dental professionals can anticipate even more sophisticated imaging modalities, real-time diagnostics, and AI-driven predictive models that enhance early detection and preventive approaches.

In conclusion, the journey of diagnostic advancements in dentistry has paved the way for a new era of precision and patient-centered care. From digital imaging to AI-powered analysis, these innovations underscore the transformative impact of technology on the diagnostic landscape, promising improved patient outcomes and a future where oral health is guided by data-driven insights and tailored interventions.

Digital Treatment Planning

In the ever-evolving landscape of modern dentistry, the advent of digital treatment planning has emerged as a transformative force, redefining the way dental procedures are conceptualized, executed, and communicated. This chapter delves into the realm of digital treatment planning, illuminating how state-of-the-art technologies and software solutions have revolutionized the process, offering dental professionals unparalleled precision, efficiency, and patient-centricity.

From Conventional to Digital Treatment Planning

Digital treatment planning represents a departure from traditional manual approaches. While conventional treatment planning relied on physical molds, sketches, and radiographs, digital methods leverage computer-assisted tools to visualize, simulate, and refine treatment strategies with remarkable accuracy.

Comprehensive Digital Data Acquisition

The foundation of digital treatment planning lies in the acquisition of comprehensive digital data. Intraoral scanners, cone beam computed tomography (CBCT), and digital radiography create a digital blueprint of a patient's oral anatomy, capturing intricate details that serve as the basis for precise treatment planning.

Virtual Simulation and Treatment Visualization

Digital treatment planning introduces the concept of virtual simulation, where treatment scenarios can be visualized and adjusted in a virtual environment. This allows dental professionals to assess different approaches, anticipate challenges, and collaborate with patients to choose the most suitable treatment path.

Orthodontics and Restorative Dentistry

In orthodontics, digital treatment planning has led to the development of virtual orthodontic setups. Orthodontists can manipulate digital models to simulate tooth movement, guiding the design of clear aligners or traditional braces. In restorative dentistry, digital treatment planning facilitates the design of crowns, bridges, and veneers, ensuring optimal fit and aesthetics.

Implantology and Surgical Precision

Digital treatment planning has transformed implant dentistry by enabling precise surgical planning. Practitioners can virtually place implants in 3D models of a patient's jaw, considering anatomical landmarks, bone density, and prosthetic requirements. This preoperative simulation enhances the accuracy of implant placement and minimizes risks.

Collaboration and Informed Consent

Digital treatment planning fosters effective communication between dental professionals and patients. Visualizations and simulations empower patients to understand proposed treatments, facilitating informed consent and shared decision-making. Patients become active participants in their oral healthcare journey.

Efficiency and Predictability

Digital treatment planning streamlines workflows, reducing chair time and enhancing efficiency. The ability to digitally plan procedures reduces the need for adjustments during treatment, leading to predictable outcomes and minimizing the potential for errors.

The Future of Personalized Dentistry

The trajectory of digital treatment planning is poised for even greater heights. As artificial intelligence, augmented reality, and bioprinting become integrated into treatment planning workflows, the future promises a new dimension of personalized care that aligns treatments with patients' unique anatomical and physiological characteristics.

In conclusion, digital treatment planning is a testament to the integration of technology with clinical acumen. From orthodontics to implantology, this approach empowers dental professionals to craft treatments that are precise, patient-centric, and optimized for success.

The journey of digital treatment planning is an evolving narrative that underscores the potential of technology to elevate the standard of care and redefine the possibilities of modern dentistry.

Importance of Technology in Dentistry

In the ever-evolving landscape of healthcare, technology has emerged as a transformative force that is reshaping the field of dentistry in profound ways. From diagnostics and treatment planning to patient engagement and practice management, technology's role in oral healthcare cannot be overstated. This chapter delves into the immense importance of technology in dentistry, highlighting how its integration has elevated precision, efficiency, patient outcomes, and overall dental practice.

Precision in Diagnostics and Treatment

One of technology's greatest contributions to dentistry is its ability to provide unparalleled precision in diagnostics and treatment. Advanced imaging techniques such as digital radiography and cone beam computed tomography (CBCT) offer detailed insights into dental and craniofacial structures, enabling practitioners to diagnose conditions with unmatched accuracy. This precision translates into more effective treatment planning and execution.

Efficiency and Streamlined Workflows

Technology has streamlined workflows, reducing manual tasks and optimizing practice operations. Digital records, electronic health records (EHRs), and practice management software simplify administrative processes, allowing dental professionals to focus on patient care. Chairside technologies like intraoral scanners expedite data acquisition, eliminating the need for conventional molds and enhancing patient comfort.

Enhanced Treatment Planning and Simulation

Incorporating technology into treatment planning allows dental professionals to visualize and simulate procedures before they are executed. Virtual treatment simulations enable practitioners to anticipate challenges, refine approaches, and engage patients in informed decision-making. This not only enhances treatment predictability but also empowers patients to actively participate in their care.

Improved Patient Engagement

Technology bridges the gap between dental professionals and patients, fostering improved communication and engagement. Patient education software, interactive treatment visualizations, and virtual consultations empower patients to understand their conditions, treatment options, and expected outcomes. Patients become informed partners in their oral health journey.

Minimized Risk and Increased Predictability

Innovations like 3D printing and computer-guided surgery have minimized risks in dental procedures. Precision-guided tools enhance the accuracy of implant placement and complex surgeries, reducing the potential for complications and postoperative issues. Predictable outcomes contribute to patient satisfaction and trust.

Access to Specialized Care

Technology has enabled greater access to specialized dental care, overcoming geographical barriers. Teledentistry allows remote consultations, enabling patients to seek expert opinions without the need for travel. This expanded access to care is especially significant for underserved populations and rural communities.

Research and Continuous Advancements

Technology catalyzes research and fosters continuous advancements in dentistry. Digital databases, AI-driven analysis, and data mining enhance the understanding of oral diseases, treatment outcomes, and preventive strategies. Dental professionals stay abreast of evolving trends and evidence-based practices, contributing to the growth of the field.

The Future: Innovation and Beyond

The importance of technology in dentistry is destined to grow. The future holds promise for even more sophisticated AI-driven diagnostics, bioprinting of dental tissues, and interconnected systems that enhance patient care and practice efficiency. The ongoing integration of technology ensures that dentistry remains at the forefront of healthcare innovation.

In conclusion, the importance of technology in dentistry is undeniable. From precision diagnostics to patient engagement and beyond, technology's impact on oral healthcare has elevated the standard of care, providing dental professionals with tools to deliver more accurate, efficient, and patient-centered treatments. As technology continues to evolve, its role in shaping the future of dentistry is destined to be transformative and empowering.

Enhanced Diagnostic Precision

In the realm of modern healthcare, the pursuit of enhanced diagnostic precision stands as a cornerstone for informed decision-making and improved patient outcomes. This chapter explores the dynamic landscape of enhanced diagnostic precision in dentistry, showcasing how cutting-edge technologies and methodologies have revolutionized the ability to identify and understand oral conditions with unparalleled accuracy and detail.

Evolution of Diagnostic Precision

Diagnostic precision has evolved from subjective assessments to data-driven insights, thanks to technology. Traditional methods often relied on visual and tactile examinations, which could be influenced by clinician experience and subjectivity. The emergence of digital tools and imaging technologies has shifted the paradigm, enabling objective and precise diagnostics.

Digital Imaging and Radiography

Digital imaging has redefined the diagnostic landscape. Digital radiography, such as intraoral and extraoral digital X-rays, offers higher resolution and dynamic range compared to traditional film radiography. This results in clearer images that aid in the detection of caries, periodontal conditions, and anomalies in hard and soft tissues.

Cone Beam Computed Tomography (CBCT)

CBCT has emerged as a game-changer in oral diagnostics. This technology provides three-dimensional images that offer a comprehensive view of dental and craniofacial structures. CBCT is invaluable for assessing impacted teeth, planning implant placements, evaluating TMJ disorders, and identifying anatomical variations with remarkable accuracy.

Advanced Imaging Techniques

Beyond traditional radiography, advanced imaging techniques like fluorescence imaging and spectroscopy have propelled diagnostic precision further. These techniques leverage the interaction of light with oral tissues to identify early signs of oral cancer and other pathologies, enabling earlier intervention and better prognoses.

Artificial Intelligence (AI) and Machine Learning

AI has taken diagnostic precision to unprecedented levels. AI algorithms can analyze vast datasets of medical images, identifying patterns and anomalies that may not be readily apparent to human clinicians. This enables early detection of conditions and the potential for predictive modeling to assess risks and outcomes.

Tailored Treatment Planning

Enhanced diagnostic precision directly influences treatment planning. With more accurate insights into a patient's condition, clinicians can develop tailored treatment plans that address individual needs and minimize unnecessary interventions. Precise diagnostics allow for more conservative and effective approaches.

Patient-Centered Outcomes

The impact of enhanced diagnostic precision extends to patient-centered outcomes. Early detection of conditions leads to timely intervention, reducing the potential for complications and more invasive treatments. Patients benefit from more efficient and effective care, resulting in improved oral health and overall well-being.

The Unfolding Future

The trajectory of enhanced diagnostic precision is bound for continued innovation. As AI continues to evolve, diagnostics will become more refined and predictive, contributing to a future where oral conditions are detected at their earliest stages, treated with precision, and ultimately preventing disease progression.

In conclusion, the pursuit of enhanced diagnostic precision in dentistry epitomizes the harmonious integration of technology, clinical acumen, and patient-centered care. From digital imaging to AI-driven analysis, these advancements not only refine diagnostics but also usher in an era where early intervention and tailored treatments become the norm, ultimately transforming the landscape of oral healthcare for the betterment of patients worldwide.

Efficiency and Streamlined Workflows

Efficiency and streamlined workflows are the bedrock upon which modern dental practice management thrives. In an era where time is of the essence and patient-centric care is paramount, the optimization of processes has become a driving force behind the evolution of oral healthcare. This chapter delves into the crucial role that efficiency and streamlined workflows play in dental practice management, highlighting how technology and innovative approaches have revolutionized the way dental professionals operate, from appointment scheduling to patient care delivery.

From Manual to Digital: A Paradigm Shift

The transition from manual to digital processes marks a pivotal paradigm shift in dental practice management. Traditional paper-based systems for patient records, appointment scheduling, and treatment planning have given way to digital solutions that offer real-time accessibility, data security, and seamless integration.

Electronic Health Records (EHRs)

EHRs have emerged as the backbone of efficient practice management. Digital patient records encompass medical histories, treatment plans, prescriptions, and more. This digital repository not only saves physical space but also streamlines record retrieval, enabling comprehensive patient care and informed decision-making.

Appointment Scheduling and Communication

Digital appointment scheduling platforms empower patients to book appointments at their convenience. Automated reminders via text messages or emails reduce no-shows, ensuring optimal use of chair time and enhancing patient engagement.

Intraoral Scanning and Impressions

Intraoral scanners have transformed the acquisition of dental impressions. The digital capture of oral anatomy eliminates the discomfort and time associated with traditional molds. Digital impressions enhance accuracy, reduce patient discomfort, and expedite the creation of restorations.

Virtual Treatment Planning

Digital workflows enable virtual treatment planning, fostering collaboration among dental professionals and providing patients with visualizations of proposed procedures. This streamlined approach ensures clarity in treatment plans, reducing the potential for misunderstandings or miscommunications.

Practice Analytics and Performance Assessment

Digital practice management systems provide insights into practice performance through analytics. Metrics like patient flow, appointment duration, and treatment success rates offer valuable data for informed decision-making and continuous improvement.

Patient-Centric Care and Engagement

Efficiency-driven workflows directly impact patient experiences. Streamlined processes minimize wait times, enhance appointment punctuality, and enable focused interactions between dental professionals and patients. Patient-centric care becomes a reality, fostering trust and loyalty.

Telehealth and Teledentistry

Advancements in telehealth and teledentistry further streamline workflows by enabling remote consultations and follow-ups. Patients can seek advice and guidance without the need for in-person visits, saving time and resources.

Future Horizons: A Seamless Practice Landscape

Efficiency and streamlined workflows are poised for continued advancement. The integration of artificial intelligence, blockchain, and predictive analytics promises an even more seamless

practice management landscape. Automated tasks, data-driven insights, and personalized patient experiences will define the future of dental practice management.

In conclusion, efficiency and streamlined workflows epitomize the synergy of technology and practice management. From electronic health records to patient engagement platforms, these advancements optimize every facet of dental operations. The journey of efficiency-driven practice management is an ongoing narrative that underscores the commitment of dental professionals to provide exceptional care while embracing the transformative potential of technology.

Enhanced Patient Experience

In the realm of modern healthcare, the concept of the patient experience has transcended mere medical care to encompass a holistic journey that intertwines clinical excellence with comfort, communication, and compassion. This chapter delves into the profound impact of enhanced patient experience in dentistry, exploring how technology has become a pivotal instrument in orchestrating empathy, communication, and personalized care that extends beyond the treatment chair.

Empathy Amplified by Technology

Technology has redefined the ways in which dental professionals express empathy toward their patients. Digital communication platforms, patient education software, and interactive visualizations enable practitioners to bridge the communication gap, fostering an environment where patients feel understood, heard, and valued.

Virtual Consultations and Teledentistry

Virtual consultations have emerged as a transformative tool to enhance patient experience. Through video conferencing and teledentistry, patients can seek advice, discuss concerns, and receive guidance without the need for physical visits. This technology-driven approach saves time, offers convenience, and expands access to care.

Personalized Education and Communication

Patient education has evolved from static brochures to dynamic digital platforms. Visual aids, animations, and interactive presentations empower patients to comprehend their conditions, treatment options, and oral health goals. Personalized communication builds trust and fosters informed decision-making.

Treatment Visualizations and Informed Decisions

Digital treatment planning brings procedures to life through visualizations. Patients can see the projected outcomes of treatments, allowing them to grasp the process and results before committing. Informed decisions lead to higher patient satisfaction and engagement.

Real-Time Updates and Convenience

Technology enables real-time updates and notifications, enhancing patient convenience. Automated appointment reminders, prescription renewals, and post-treatment instructions keep patients engaged and informed, reducing the likelihood of missed appointments and misunderstandings.

Minimized Discomfort and Anxiety

Technological innovations contribute to reducing patient discomfort and anxiety. Intraoral scanners replace traditional molds, eliminating the sensation of gagging. Virtual reality and relaxation apps provide distraction and reduce anxiety during procedures.

Patient Portals and Engagement Platforms

Patient portals provide a centralized platform for patients to access their records, treatment plans, and educational resources. These portals encourage proactive engagement, enabling patients to take charge of their oral health and collaborate in their care.

The Healing Power of Communication

Technology facilitates ongoing communication between appointments. Email follow-ups, treatment progress updates, and postoperative care instructions demonstrate ongoing support, reassuring patients and facilitating a sense of continuity in their care journey.

The Future: Tailored Experiences

The journey of enhancing patient experience through technology is an ever-evolving one. As artificial intelligence, wearables, and personalized treatment algorithms become integrated, the future promises even more tailored experiences that consider patients' unique preferences, fears, and aspirations.

In conclusion, the orchestration of enhanced patient experience through technology underscores the harmonious integration of compassionate care and technological innovation. From virtual consultations to personalized education, these advancements empower patients to take an active role in their oral health while fostering trust, loyalty, and a renewed sense of partnership between dental professionals and those they serve.

Financial Considerations and Cost Efficiency

While the initial investment in digital equipment and software might seem substantial, the long-term benefits often outweigh the costs. Digital dentistry reduces the need for physical

storage of records and eliminates the expenses associated with traditional impression materials. Moreover, the efficiency gained through streamlined workflows allows for increased patient throughput, potentially leading to higher revenue generation.

Conclusion

Chapter 1 provides a foundational understanding of the evolution of digital dentistry and its pivotal role in contemporary oral healthcare. From its humble beginnings in the realm of dental imaging to its current status as a catalyst for enhanced diagnostics and treatment planning, digital dentistry continues to shape the future of dentistry. The integration of technology brings forth a new era of precision, efficiency, and patient-centric care, setting the stage for the exploration of the exciting trends and innovations covered in the subsequent chapters of this book.

Chapter 2: Digital Imaging and Diagnostic Techniques

3D Imaging and Cone Beam Computed Tomography

In the dynamic landscape of dental diagnostics, the advent of 3D imaging and Cone Beam Computed Tomography (CBCT) has ushered in a new era of precision, accuracy, and comprehensive understanding of oral and maxillofacial structures. This chapter delves into the remarkable world of 3D imaging and CBCT, highlighting their transformative impact on diagnosis, treatment planning, and patient care in the realm of dentistry.

The Evolution of Imaging: From 2D to 3D

Traditionally, dental diagnostics relied on two-dimensional (2D) imaging techniques such as panoramic radiographs and bitewings. While valuable, these methods presented limitations in terms of depth perception and comprehensive visualization. 3D imaging emerged as a solution to overcome these limitations, offering a volumetric view of dental structures that revolutionized the diagnostic landscape.

Cone Beam Computed Tomography (CBCT): A Leap Forward

At the heart of 3D imaging lies CBCT, a technology that utilizes a cone-shaped X-ray beam to capture multiple cross-sectional images of the oral and maxillofacial region. These images are then reconstructed into a three-dimensional representation, providing a comprehensive view of dental anatomy, bone structure, soft tissues, and anatomical relationships.

Clinical Applications and Impact

The applications of 3D imaging and CBCT span a wide spectrum of dental specialties, each benefiting from the unprecedented insights these technologies offer:

Implant Dentistry: CBCT enables precise preoperative assessment of bone density, anatomical structures, and optimal implant placement sites. This results in accurate planning and successful implant outcomes.

Orthodontics: 3D imaging aids in diagnosing skeletal discrepancies, assessing root positions, and planning orthodontic treatments. Virtual simulations assist orthodontists in predicting tooth movement and guiding treatment strategies.

Oral Surgery: CBCT facilitates meticulous evaluation of impacted teeth, cysts, tumors, and pathologies. Precise localization minimizes risks during surgical interventions.

Endodontics: 3D imaging aids in identifying complex root canal anatomy, diagnosing periapical lesions, and guiding endodontic procedures.

TMJ Evaluation: CBCT provides detailed insights into temporomandibular joint (TMJ) anatomy, facilitating diagnosis and treatment planning for TMJ disorders.

Benefits and Advantages

The benefits of 3D imaging and CBCT are manifold:

Precision: 3D images offer a higher level of precision and accuracy compared to 2D images, enabling clinicians to detect subtle pathologies and plan treatments with greater confidence.

Comprehensive Evaluation: CBCT offers a holistic view of dental and facial structures, providing insights into anatomical relationships, bone density, and the presence of pathologies.

Minimized Radiation Exposure: While CBCT involves radiation, its focused beam and rapid image acquisition minimize radiation exposure compared to traditional medical CT scans.

Enhanced Treatment Planning: 3D images facilitate virtual treatment planning, allowing clinicians to simulate procedures, anticipate challenges, and engage patients in informed decision-making.

Limitations and Responsibility

While the advantages are clear, responsible use of CBCT is essential due to the associated radiation exposure. Clinicians must adhere to appropriate clinical guidelines and only use CBCT when the benefits outweigh the risks. Ethical practice involves discussing the benefits, risks, and alternatives with patients before performing a CBCT scan.

Future Frontiers: Unveiling New Horizons

As technology evolves, so do the possibilities of 3D imaging and CBCT. Integration with artificial intelligence, advanced image analysis, and improved accessibility promise to reshape the diagnostic landscape further, enhancing both clinical accuracy and patient experiences.

In conclusion, 3D imaging and CBCT represent a watershed moment in dental diagnostics, enabling practitioners to explore the depth of oral structures and anomalies with unprecedented clarity. From implantology to orthodontics, these technologies exemplify the

transformative power of precision in guiding treatment strategies and elevating patient care in the realm of dentistry.

Unveiling 3D Imaging

In the realm of dental diagnostics, the introduction of 3D imaging has ignited a revolutionary shift in the way oral structures are visualized and understood. This chapter unveils the intricacies of 3D imaging, shedding light on its transformative impact on dentistry by providing a comprehensive view of dental anatomy, facilitating accurate diagnoses, guiding treatment strategies, and elevating patient care to new heights.

Entering the Third Dimension

3D imaging marks a departure from traditional two-dimensional radiography, offering a volumetric perspective that unveils the intricate details of dental and maxillofacial structures. Unlike conventional X-rays, which flatten images into a single plane, 3D imaging captures cross-sectional views that allow clinicians to explore structures from multiple angles.

The Science Behind 3D Imaging

The cornerstone of 3D imaging lies in its technology, with Cone Beam Computed Tomography (CBCT) being a prominent example. CBCT employs a cone-shaped X-ray beam that rotates around the patient, capturing a series of images that are reconstructed into a three-

dimensional representation. This technique provides a layer-by-layer view of oral structures, offering unprecedented insights.

Clinical Applications and Advantages

The applications of 3D imaging in dentistry are far-reaching:

Implant Planning: 3D imaging revolutionizes implant dentistry by enabling precise assessment of bone density, anatomical landmarks, and optimal implant placement sites. Clinicians can visualize the available bone volume, avoiding critical structures and ensuring successful outcomes.

Endodontics: In endodontics, 3D imaging aids in diagnosing complex root canal anatomy, identifying hidden canals, and assessing treatment outcomes with enhanced accuracy.

Oral and Maxillofacial Surgery: The technology assists in the assessment of impacted teeth, planning of surgical procedures, and diagnosing conditions such as cysts and tumors with unparalleled clarity.

Orthodontics: Orthodontists benefit from 3D imaging's ability to visualize skeletal relationships, assess root positions, and simulate tooth movement, leading to more precise treatment planning.

TMJ Evaluation: 3D imaging provides insights into temporomandibular joint (TMJ) anatomy, aiding in the diagnosis and management of TMJ disorders.

Patient Education and Informed Consent: 3D images empower patients to understand their conditions visually, facilitating informed decision-making and improving patientengagement.

Challenges and Considerations

While 3D imaging offers a wealth of advantages, responsible use is paramount. The technology involves radiation exposure, albeit at a lower dose than traditional medical CT scans. Clinicians must adhere to ethical guidelines and clinical protocols to ensure patient safety and only use 3D imaging when clinically justified.

Charting the Future: Advancements Await

As technology continues to evolve, so too will the capabilities of 3D imaging. Integration with artificial intelligence, automated analysis tools, and improved accessibility will redefine the diagnostic landscape, enhancing both clinical outcomes and patient experiences.

In conclusion, 3D imaging unveils the hidden dimensions of oral anatomy, transforming dentistry's diagnostic capabilities. From precise implant planning to comprehensive surgical assessments, these technologies embody the fusion of clinical expertise and cutting-edge

innovation. As dentistry journeys into the realm of three-dimensional insights, the future promises an era of unprecedented accuracy, improved patient care, and a deeper understanding of the complexities of oral health.

Enhanced Diagnostics

In the ever-evolving landscape of healthcare, enhanced diagnostics have emerged as a beacon of accuracy, guiding clinicians toward precise understanding and informed decision-making. This chapter delves into the realm of enhanced diagnostics in dentistry, spotlighting how advanced technologies, innovative techniques, and data-driven approaches have elevated the capacity to identify, assess, and comprehend oral conditions with unprecedented precision.

Advancing Beyond Conventional Diagnostics

Enhanced diagnostics transcend traditional methods, introducing a new era of data-rich insights:

Digital Radiography: Digital X-rays replace film-based radiography, offering clearer images, reduced radiation exposure, and immediate availability for review.

Intraoral Scanners: These devices digitally capture detailed impressions of teeth and soft tissues, replacing conventional molds and enhancing patient comfort.

Fluorescence Imaging: Specialized imaging techniques utilize fluorescent agents to highlight early signs of oral diseases, such as caries or oral cancer.

Cone Beam Computed Tomography (CBCT): This three-dimensional imaging technique provides detailed cross-sectional views of oral structures, enabling comprehensive assessments.

Salivary Diagnostics: Emerging technologies allow the analysis of saliva for biomarkers indicative of oral and systemic health conditions.

The Power of Data and Digitalization

Enhanced diagnostics harness the potential of data-driven insights:

Computer-Aided Analysis: Advanced software and artificial intelligence algorithms analyze radiographic images, identifying patterns, anomalies, and risk factors that might elude human observation.

Predictive Analytics: Data-driven models anticipate disease progression and treatment outcomes, facilitating early intervention and personalized care.

Telemedicine and Teledentistry: Digital platforms enable remote consultations, bringing expert opinions to underserved populations and enhancing access to specialized care.

Impact on Treatment Planning and Execution

Enhanced diagnostics profoundly influence treatment approaches:

Precision Treatment Planning: Accurate diagnostic data inform personalized treatment plans, optimizing outcomes and minimizing the need for adjustments.

Minimized Invasiveness: Precise diagnostics aid in minimally invasive procedures, preserving healthy tissue while addressing conditions accurately.

Implant Dentistry: Enhanced diagnostics ensure optimal implant placement, reducing risks and enhancing the longevity of prosthetic restorations.

Orthodontics: Accurate assessments guide orthodontic treatment plans, promoting efficient tooth movement and reduced treatment duration.

Early Detection and Prevention

Enhanced diagnostics excel in early detection:

Oral Cancer: Advanced imaging techniques detect subtle changes, enabling earlier diagnosis and improving survival rates.

Caries Detection: Enhanced diagnostics identify cavities in their earliest stages, enabling less invasive interventions.

Periodontal Disease: Precise assessments facilitate timely periodontal disease management, preventing irreversible damage.

Looking Ahead: The Future of Enhanced Diagnostics

The journey of enhanced diagnostics is one of continuous evolution:

Nanotechnology: Nanoscale imaging and diagnostic tools promise unparalleled sensitivity and specificity.

Biological Markers: Emerging biomarkers offer insights into early disease detection and treatment response.

Genomic Diagnostics: Genetic data informs disease predisposition and personalized treatment strategies.

In conclusion, enhanced diagnostics represent the convergence of technological prowess and clinical acumen. From accurate assessments to informed treatment decisions, these advancements herald a new era where patient care is not just reactive but proactive, aiming to prevent, detect, and manage oral health conditions with unparalleled precision.

Implant Planning and Placement

In the landscape of modern restorative dentistry, the art and science of implant planning and placement have undergone a transformative evolution. This chapter unveils the intricacies of implant planning and placement, illuminating how advanced technologies, meticulous diagnostics, and interdisciplinary collaboration have redefined the landscape of dental implantology, enabling clinicians to restore function, aesthetics, and patient confidence with unmatched precision.

The Essence of Dental Implantology

Dental implants represent a cornerstone of modern dentistry, offering a solution for restoring missing teeth that goes beyond conventional prosthetics. Implant planning and placement

involve a harmonious blend of clinical expertise, meticulous assessment, and technological innovation, all aimed at achieving a natural, functional, and long-lasting outcome.

Precise Diagnostics: The Foundation

Implant planning begins with comprehensive diagnostics:

Cone Beam Computed Tomography (CBCT): 3D imaging technology provides detailed views of bone density, anatomical landmarks, and adjacent structures, enabling accurate assessment for optimal implant placement.

Digital Impressions: Intraoral scanners capture precise digital impressions, aiding in the design and fabrication of customized implant restorations.

Treatment Goals and Aesthetics: Patient consultation involves understanding treatment goals, desired aesthetics, and expectations, allowing for a personalized treatment plan.

Navigating the Virtual Realm: Virtual Treatment Planning

Technology-driven virtual treatment planning brings implantology to life:

Computer-Aided Design (CAD): CAD software allows for precise digital implant placement, taking into account anatomical factors, restorative needs, and aesthetics.

Simulated Treatment: Virtual simulations provide clinicians and patients with a visual representation of the proposed treatment outcome, facilitating informed decision-making.

Interdisciplinary Collaboration: Communication between restorative dentists, oral surgeons, and prosthodontists ensures a cohesive treatment approach, optimizing implant outcomes.

Navigational Tools: Guided Implant Surgery

Guided implant surgery enhances precision and predictability:

Surgical Guides: 3D-printed surgical guides transfer virtual plans to the surgical site, ensuring precise implant placement according to the treatment plan.

Minimized Invasiveness: Guided surgery reduces the need for flap elevation, promoting faster healing, reduced postoperative discomfort, and minimal tissue trauma.

Immediate Loading: Guided implant surgery often enables immediate loading, where a temporary restoration can be attached to the implant shortly after placement, enhancing patient comfort and function.

Success Markers: Osseointegration and Restoration

The success of implant planning and placement extends to osseointegration and restoration:

Osseointegration: Implants integrate with the surrounding bone, providing stability for prosthetic restorations and mimicking the function of natural teeth.

Prosthetic Restoration: Customized restorations, such as crowns, bridges, or dentures, are fabricated to seamlessly blend with the patient's natural dentition, restoring aesthetics and function.

Follow-Up and Maintenance: Regular follow-up appointments and proper maintenance ensure the longevity of implant restorations, contributing to patient satisfaction.

The Future: Tailored Solutions and Beyond

As technology advances, the future of implant planning holds exciting prospects:

Biomimetic Implants: Implants designed to mimic natural tooth structure enhance aesthetics and function.

Digital Impressions: Evolving technologies may eliminate the need for physical impressions entirely, enhancing patient comfort and convenience.

In conclusion, implant planning and placement embody the synergy of art and science. From meticulous diagnostics to precision-guided surgery, these advancements exemplify dentistry's commitment to achieving excellence in functional and aesthetic outcomes. As the landscape of implantology continues to evolve, the future promises personalized solutions that redefine the possibilities of dental restoration, offering patients the confidence to smile, speak, and chew with renewed vitality.

Orthodontic Treatment Planning

Orthodontic treatment has also benefited significantly from 3D imaging. CBCT scans provide orthodontists with detailed insights into skeletal relationships, tooth angulations, and eruption patterns. This information is vital for devising customized treatment plans, especially in complex cases involving impacted teeth, malocclusions, and orthognathic surgery.

Intraoral Scanning and Digital Impressions

The Digital Impression Revolution

Traditional dental impressions, often associated with discomfort and inaccuracies, have been transformed by the advent of intraoral scanning technology. This section explores how digital impressions have redefined the way dental professionals capture oral structures, streamlining workflows and enhancing patient experiences.

Intraoral Scanners: How They Work

Intraoral scanners use optical technology to capture precise digital impressions of the patient's oral cavity. The scanner projects a light pattern onto the teeth and surrounding tissues, while a camera captures the reflection. Advanced software then processes these images to create a highly accurate 3D representation of the patient's dentition.

Benefits of Digital Impressions

Digital impressions offer numerous advantages over traditional methods. Firstly, they eliminate the need for uncomfortable impression materials, reducing patient anxiety and discomfort. Additionally, digital impressions provide immediate feedback, ensuring that scans are complete and accurate before the patient leaves the chair. The elimination of physical models also reduces storage requirements and allows for seamless electronic sharing of data with laboratories and specialists.

Applications in Restorative Dentistry

Intraoral scanning technology has revolutionized restorative dentistry by expediting the process of crown, bridge, and veneer fabrication. Digital impressions are sent directly to computer-aided design (CAD) software, where dental professionals can design custom restorations with unprecedented precision. This streamlined workflow reduces turnaround times, enabling patients to receive their final restorations more quickly.

Orthodontics and Aligner Therapy

In the realm of orthodontics, digital impressions have become a cornerstone of treatment planning. Orthodontists can use digital models to visualize tooth movements, simulate treatment outcomes, and design customized aligners. This technology-driven approach not only enhances treatment accuracy but also improves patient compliance and satisfaction.

Conclusion

Chapter 2 illuminates the revolutionary impact of 3D imaging and intraoral scanning on the landscape of modern dentistry. From the transformative capabilities of cone beam computed tomography to the seamless precision of digital impressions, these technologies have redefined diagnostics, treatment planning, and patient experiences. As the journey into the future of digital dentistry continues, these tools will undoubtedly play a central role in shaping the way dental professionals approach oral healthcare.

Chapter 3: Computer-Aided Design (CAD) in Dentistry

CAD/CAM Systems for Restorations

The fusion of computer-aided design (CAD) and computer-aided manufacturing (CAM) technologies has ushered in a new era of precision and efficiency in restorative dentistry. This chapter delves into the transformative impact of CAD/CAM systems on the creation of dental restorations, from crowns and bridges to veneers and inlays.

Designing in the Digital Realm CAD/CAM systems represent a paradigm shift in the way dental restorations are designed and manufactured. This section provides an in-depth exploration of the CAD process, wherein digital impressions are used to create virtual 3D models of patients' dentition. These digital models serve as the foundation for designing custom restorations with intricate precision.

Customization and Precision One of the most remarkable aspects of CAD/CAM technology is the level of customization it affords. Dental professionals can meticulously design

restorations that fit seamlessly with the patient's existing dentition. The ability to manipulate virtual models allows for precise adjustments, ensuring optimal occlusion, marginal fit, and aesthetics.

Streamlining Workflow CAD/CAM systems significantly expedite the restoration process. Once the digital design is complete, it is seamlessly transferred to the computer-aided manufacturing (CAM) component of the system. This integration enables the fabrication of restorations in a fraction of the time compared to traditional methods, reducing the number of patient visits and enhancing overall practice efficiency.

Materials and Fabrication The chapter delves into the array of materials compatible with CAD/CAM systems. From ceramics and zirconia to resin composites, these materials offer a range of aesthetic and functional options. The fabrication process involves milling or 3D printing, both of which produce highly accurate restorations that require minimal adjustments during placement.

Virtual Smile Design and Aesthetics

The integration of CAD technology extends beyond functional restorations to the realm of aesthetics. This section explores how virtual smile design tools have transformed the way clinicians and patients collaborate in achieving desired aesthetic outcomes.

Empowering Patient Engagement Virtual smile design allows patients to actively participate in the treatment planning process. Dental professionals can use software to create virtual simulations of aesthetic changes, such as tooth shape, size, and color. Patients can visualize potential results before committing to treatment, fostering informed decision-making and patient satisfaction.

Aesthetic Considerations The chapter discusses the aesthetic principles that guide virtual smile design. Factors such as tooth proportion, symmetry, gingival display, and lip line play crucial roles in achieving harmonious smile aesthetics. Dental professionals can manipulate digital models to strike the perfect balance between natural appearance and patient preferences.

Collaborative Treatment Planning Virtual smile design facilitates seamless communication between patients and clinicians. Through interactive consultations, patients can articulate their aesthetic goals, and dental professionals can provide realistic insights into what can be achieved. This collaborative approach not only enhances patient satisfaction but also helps manage expectations and ensure successful treatment outcomes.

Conclusion

Chapter 3 illuminates the impact of computer-aided design (CAD) on the creation of dental restorations and the transformation of aesthetic treatment planning. The integration of CAD technology enhances precision, customization, and collaboration in the field of dentistry. From crafting functional restorations with unmatched accuracy to empowering patients to actively engage in their aesthetic journeys, CAD/CAM systems continue to redefine the boundaries of dental care and creativity.

Chapter 4: Additive Manufacturingand 3D Printing

Applications of 3D Printing in Dentistry

The advent of 3D printing has heralded a transformative era in dentistry, elevating the possibilities of precision, customization, and innovation. This chapter delves into the multifaceted applications of 3D printing in dentistry, showcasing how this technology has revolutionized the creation of dental prosthetics, surgical guides, orthodontic devices, and more, paving the way for unparalleled patient care and treatment outcomes.

The 3D Printing Revolution

3D printing, also known as additive manufacturing, enables the layer-by-layer fabrication of complex structures from digital designs. In dentistry, this technology has emerged as a game-changer, offering solutions that were once unattainable through traditional methods.

Customized Prosthetics and Restorations

Dental Crowns and Bridges: 3D printing allows for the fabrication of precise and customized dental crowns and bridges, ensuring optimal fit, function, and aesthetics.

Dentures: Removable dentures can be crafted using 3D printing, providing patients with comfortable and well-fitting prosthetics.

Implant Abutments: Customized implant abutments can be 3D printed to match the patient's unique anatomical requirements, enhancing the stability and aesthetics of implant restorations.

Orthodontic Solutions

Clear Aligners: 3D printing has transformed orthodontics through the creation of clear aligners. These removable trays offer a discreet and effective solution for tooth alignment.

Orthodontic Models: 3D printed models of patients' teeth aid in treatment planning and the creation of orthodontic appliances.

Surgical Precision: Guides and Models

Surgical Guides: 3D printed surgical guides ensure precise implant placement, optimizing the success of implant procedures and minimizing invasiveness.

Anatomical Models: 3D printed anatomical models help clinicians visualize complex cases, facilitating treatment planning and patient communication.

Educational Tools and Patient Engagement

Demonstration Models: 3D printed models aid in explaining treatment plans to patients, enhancing their understanding and engagement.

Hands-On Training: Dental students and professionals can practice procedures on accurate 3D printed models, improving their skills before performing procedures on patients.

Restorative Aesthetics and Beyond

Smile Design: 3D printing assists in smile design by creating wax-ups and mock-ups that allow patients to preview and approve aesthetic changes.

Surgical Provisions: Temporary restorations and surgical splints can be 3D printed, aiding in immediate loading procedures and postoperative comfort.

Future Horizons: Bioprinting and Beyond

The future of 3D printing in dentistry holds promise for even more advanced applications:

Bioprinting: The field of bioprinting aims to create living tissues and organs using 3D printing technology, with potential implications for dental tissue engineering.

Material Innovations: Advances in 3D printing materials continue to expand the range of possibilities, with biocompatible and durable materials driving innovation.

In conclusion, the applications of 3D printing in dentistry epitomize the fusion of technology and artistry. From crafting customized prosthetics to guiding intricate surgeries, this technology has empowered clinicians to transcend previous limitations and offer patients treatment solutions that are precise, comfortable, and tailored to their individual needs. The journey of 3D printing in dentistry continues to shape the future of patient care, promising a realm of endless possibilities for enhanced treatment outcomes and patient satisfaction.

Understanding Additive Manufacturing

In the realm of modern manufacturing, additive manufacturing, commonly known as 3D printing, has emerged as a revolutionary technique that is reshaping industries across the spectrum. This chapter delves into the essence of additive manufacturing, exploring its

fundamental principles, processes, and the transformative impact it has had on diverse fields, from aerospace to healthcare, with a particular focus on dentistry.

The Essence of Additive Manufacturing

At its core, additive manufacturing is a technique that involves creating three-dimensional objects by adding material layer by layer, in contrast to subtractive manufacturing, which involves cutting away material from a larger piece. This layered approach has revolutionized the manufacturing landscape, enabling the creation of intricate geometries and designs that were previously unattainable.

The Additive Manufacturing Process

The additive manufacturing process typically involves the following steps:

1. **Digital Design:** A digital 3D model of the desired object is created using computer-aided design (CAD) software.
2. **Slicing:** The 3D model is divided into thin horizontal layers using slicing software, which generates instructions for the 3D printer.
3. **Printing:** The 3D printer follows the instructions from the slicing software, adding material layer by layer to gradually build the object.
4. **Post-Processing:** Depending on the material and application, post-processing steps such as curing, polishing, or painting may be required to achieve the desired finish.

Applications Across Industries

Additive manufacturing has transcended traditional manufacturing methods in various industries:

Aerospace: 3D printing is used to create lightweight and complex parts for aircraft and spacecraft, optimizing performance and reducing weight.

Automotive: Prototyping, custom parts, and tooling are efficiently produced using additive manufacturing.

Healthcare: In dentistry and medical fields, additive manufacturing has revolutionized prosthetics, implants, surgical tools, and anatomical models.

Art and Design: Creative professionals use 3D printing to bring intricate and imaginative designs to life.

Consumer Goods: Customizable consumer products, from jewelry to fashion accessories, can be produced using additive manufacturing.

The Role of Additive Manufacturing in Dentistry

In the realm of dentistry, additive manufacturing has catalyzed transformative changes:

Prosthetics and Restorations: 3D printing enables the creation of precise and customized dental crowns, bridges, dentures, and implants.

Surgical Guides: Additive manufacturing produces surgical guides that aid in precise implant placement and oral surgeries.

Orthodontics: Clear aligners, orthodontic models, and customized appliances are manufactured with accuracy using 3D printing.

Educational Tools: Anatomical models and demonstration tools enhance patient communication and education.

Future Vistas: Advancements in Materials and Bioprinting

The future of additive manufacturing holds exciting possibilities:

Advanced Materials: The development of new materials, including biocompatible options, is expanding the range of applications.

Bioprinting: The field of bioprinting aims to create living tissues and organs using 3D printing technology, with implications for regenerative dentistry.

In conclusion, additive manufacturing stands as a transformative force that has propelled industries into a new era of innovation and customization. In dentistry, the layer-by-layer construction of intricate dental solutions showcases the potential for additive manufacturing to elevate patient care, offering personalized and precise treatment outcomes that were once beyond imagination. As the technology continues to evolve, the horizon of possibilities broadens, promising a future where additive manufacturing reshapes industries and healthcare alike.

Printed Dental Prosthetics

In the realm of restorative dentistry, the advent of additive manufacturing, also known as 3D printing, has ushered in a new era of possibilities for crafting dental prosthetics with unparalleled precision, aesthetics, and patient-centric design. This chapter delves into the realm of printed dental prosthetics, showcasing how this technology has redefined the creation of dental crowns, bridges, dentures, and implants, revolutionizing patient care and enhancing smiles with meticulous detail.

A Paradigm Shift in Prosthetic Fabrication

Traditional methods of fabricating dental prosthetics often involved labor-intensive processes, intricate casting, and a degree of variability. Additive manufacturing has revolutionized this landscape, offering a streamlined and digital approach that brings precision and customization to the forefront.

Printed Dental Crowns and Bridges

Customization: 3D printing enables the creation of dental crowns and bridges that are tailored to each patient's unique anatomy, ensuring optimal fit and aesthetics.

Digital Workflow: From digital impressions to computer-aided design (CAD) and 3D printing, the entire workflow is streamlined, reducing the time and effort required for fabrication.

Material Selection: A range of biocompatible materials, including ceramics and resin composites, can be used for printing, catering to various clinical needs.

Implant Restorations

Personalized Abutments: Additive manufacturing allows for the creation of implant abutments that perfectly match the patient's anatomy, ensuring stability and longevity.

Implant-Supported Dentures: Dentures supported by implants can be 3D printed, enhancing comfort, stability, and chewing efficiency.

Clear Aligners and Orthodontic Appliances

Custom Clear Aligners: 3D printing has transformed orthodontics by enabling the creation of clear aligners that gradually align teeth discreetly.

Orthodontic Models: Accurate 3D printed models facilitate orthodontic treatment planning and the creation of customized appliances.

Digital Smile Design

Aesthetic Preview: Patients can visualize their future smiles through 3D-printed mock-ups, ensuring their preferences are met before final prosthetics are fabricated.

Sculpting Confidence: Patient Benefits

Precise Fit: Printed prosthetics fit with unparalleled accuracy, reducing the need for adjustments and improving patient comfort.

Efficiency: The digital workflow expedites the fabrication process, reducing chair time and increasing patient satisfaction.

Aesthetics: Printed prosthetics mimic natural teeth, offering aesthetics that blend seamlessly with the patient's dentition.

Minimized Invasiveness: Accurate fit and minimal adjustments result in less invasive procedures, preserving healthy tooth structure.

Looking Ahead: Future Prospects

As additive manufacturing continues to evolve, the future of printed dental prosthetics holds exciting possibilities:

Biocompatible Materials: Advances in materials science will expand the range of biocompatible and durable materials suitable for printing.

Bioprinting: The field of bioprinting may pave the way for printing living tissues and even tooth structures in the future.

Conclusion: Sculpting Smiles with Precision

Printed dental prosthetics epitomize the convergence of technology and artistry. From digital design to precise fabrication, this technology allows dental professionals to craft restorations that blend functionality, aesthetics, and patient preferences seamlessly. As 3D printing advances, the journey of printed dental prosthetics continues to shape the future of restorative

dentistry, offering patients renewed confidence, comfort, and smiles that stand as testaments to precision and care.

Orthodontics and Clear Aligners

In the dynamic realm of orthodontics, the introduction of clear aligners has sparked a revolution, transforming the way teeth alignment is achieved. This chapter delves into the world of orthodontics and clear aligners, shedding light on how these innovative solutions have redefined the path to well-aligned smiles, offering patients discreet treatment options, enhanced comfort, and improved aesthetics.

A Paradigm Shift in Orthodontics

Traditionally, orthodontic treatment often involved braces – metallic brackets and wires – to correct dental misalignments. Clear aligners, an innovation rooted in advanced technology, have changed the landscape of orthodontics by offering a more aesthetically pleasing, comfortable, and patient-centric approach to teeth alignment.

The Essence of Clear Aligners

Clear aligners are custom-made, removable trays that gradually guide teeth into their desired positions. This technology embodies precision and personalization, offering a discreet alternative to traditional braces.

The Journey: Orthodontics with Clear Aligners

Digital Impressions: The orthodontic journey begins with digital impressions, where 3D scans of the patient's teeth are captured using intraoral scanners.

Treatment Planning: Orthodontists use computer-aided design (CAD) software to plan the step-by-step movement of teeth, designing a series of aligners that will guide teeth into alignment.

Custom Fabrication: Each set of aligners is 3D printed or thermoformed based on the digital treatment plan. Aligners are typically worn for one to two weeks before progressing to the next set.

Patient Experience and Benefits

Discreet Appearance: Clear aligners are virtually invisible, enabling patients to undergo orthodontic treatment without the aesthetic impact of traditional braces.

Removability: Aligners can be removed for eating, drinking, brushing, and flossing, enhancing oral hygiene and dietary flexibility.

Enhanced Comfort: Unlike braces, aligners have no sharp brackets or wires that can cause discomfort or irritate soft tissues.

Digital Treatment Visualization: Patients can see a visual representation of their treatment journey, including the expected final result.

Predictable Outcomes: Computer-aided treatment planning ensures accurate predictions of treatment progress and outcomes.

The Power of Technology and Innovation

Advanced Software: CAD software and 3D imaging enable precise treatment planning, ensuring optimal tooth movement.

Iterative Improvement: Aligner technology continually evolves, with each generation offering enhanced comfort, fit, and treatment outcomes.

Indications and Limitations

Clear aligners are effective for various orthodontic issues, including mild to moderate crowding, spacing, and bite misalignments. However, certain complex cases or severe misalignments may still benefit from traditional braces.

The Future: Expanding Horizons

The evolution of clear aligners promises exciting prospects:

Younger Patients: As technology advances, clear aligners may become more suitable for young patients who require growth modulation.

Combined Approaches: Clear aligners may be combined with other orthodontic techniques for optimal results in complex cases.

Conclusion: Transforming Smiles with Confidence

Orthodontics with clear aligners exemplifies the marriage of innovation and aesthetics. This revolutionary approach empowers patients to embark on the journey to well-aligned smiles without the concerns of traditional braces. As technology continues to evolve, the horizon of possibilities broadens, promising a future where orthodontics remains a transformative force in enhancing smiles and boosting self-confidence for individuals of all ages.

Advancements in Implantology

In the ever-evolving landscape of dental implantology, advancements in technology, materials, and techniques have ushered in a new era of precision, predictability, and patient-centric outcomes. This chapter delves into the world of implantology, illuminating how

cutting-edge innovations have redefined the field, transforming the way dental implants are planned, placed, and restored, ultimately enhancing patient satisfaction and quality of life.

The Evolution of Implant Dentistry

Implant dentistry has witnessed a remarkable journey from its inception to the present day. While the fundamental principles remain unchanged – the anchoring of artificial tooth roots within the jawbone – the methods and tools employed have undergone transformative advancements that have elevated the field to new heights.

Digital Treatment Planning: The Blueprint for Success

Cone Beam Computed Tomography (CBCT): Three-dimensional imaging has revolutionized treatment planning, providing detailed insights into bone density, anatomical landmarks, and adjacent structures.

Computer-Aided Design (CAD) and Computer-Aided Manufacturing (CAM): CAD/CAM technology facilitates the design and fabrication of implant restorations with precision, ensuring optimal fit and aesthetics.

Virtual Simulations: Clinicians can virtually plan implant placement, anticipate challenges, and visualize treatment outcomes before initiating procedures.

Guided Implant Surgery: Guided surgery involves the use of surgical guides created through 3D printing or milling, ensuring precise implant placement based on virtual treatment plans.

Implant Materials and Surface Innovations

Titanium and Beyond: Titanium implants remain the gold standard, offering biocompatibility and osseointegration. Advancements include enhanced surface modifications to accelerate healing.

Zirconia Implants: Ceramic zirconia implants have gained popularity due to their tooth-like appearance and biocompatibility, catering to patients with aesthetic concerns.

Regenerative Techniques: Growth factors, bone grafting materials, and tissue engineering approaches enhance bone and soft tissue regeneration, ensuring optimal implant stability.

Immediate Loading and Same-Day Implants

Advancements in implantology have led to same-day implant placement and restoration, offering patients a rapid solution for missing teeth without the need for multiple appointments.

Enhancing Aesthetics and Functionality

Customized Abutments: CAD/CAM technology enables the creation of customized abutments that match the patient's anatomical contours and achieve optimal emergence profiles.

Digital Smile Design: Implant placement can be planned based on the desired final aesthetic outcome, ensuring seamless integration with the patient's natural dentition.

Prosthetic Solutions: Innovations in prosthetic design and materials enable the creation of implant-supported restorations that mimic natural teeth in form and function.

Patient-Centric Care and Outcomes

Minimized Invasiveness: Advancements in surgical techniques lead to minimally invasive procedures, reducing postoperative discomfort and enhancing healing.

Predictable Outcomes: Digital treatment planning and guided surgery enhance predictability, reducing the risk of complications and ensuring successful outcomes.

Patient Education: Advanced visualization tools enable patients to understand their treatment plans, fostering informed decision-making and participation.

The Path Ahead: Expanding Horizons

As implantology continues to advance, the future holds exciting possibilities:

Biological Integration: Research into enhancing the biological integration of implants promises to further optimize long-term success rates.

Personalized Implantology: Tailored treatment plans based on genetic and anatomical factors will revolutionize patient care.

Conclusion: Precision Redefined, Smiles Enhanced

Advancements in implantology epitomize the marriage of science, technology, and artistry. The journey from treatment planning to restoration represents a symphony of innovation that ensures the success and satisfaction of patients seeking functional, aesthetic, and enduring solutions for missing teeth. As the field continues to progress, implantology will remain at the forefront of dental innovation, reshaping smiles and enhancing lives.

Materials Used in Dental 3D Printing

Materials Landscape The chapter shifts its focus to the materials landscape of dental 3D printing. From polymers and ceramics to metals, a diverse range of materials is now available for additive manufacturing in dentistry. This section delves into the characteristics and

applications of various materials, elucidating how each material's properties align with specific dental applications.

Biocompatibility and Performance The selection of appropriate materials is crucial in ensuring biocompatibility and long-term performance of 3D-printed dental devices. The chapter explores the compatibility of different materials with oral tissues, highlighting the importance of material choices in restorative dentistry, prosthetics, and orthodontics.

Challenges and Future Prospects

Overcoming Challenges While 3D printing holds immense promise, it is not devoid of challenges. This section addresses considerations such as material compatibility, accuracy, and post-processing requirements. It also discusses the regulatory landscape surrounding 3D-printed medical devices and the need for standardized protocols to ensure patient safety.

Future Innovations The chapter concludes by shedding light on the future potential of 3D printing in dentistry. From bioprinting of functional dental tissues to the customization of drug delivery systems, the possibilities are vast. The integration of 3D printing with other digital technologies, such as artificial intelligence and virtual reality, further expands its horizons.

Conclusion

Chapter 4 unveils the transformative power of additive manufacturing and 3D printing within the field of dentistry. By showcasing applications ranging from dental prosthetics to orthodontic aligners and implantology, the chapter highlights how this technology is reshaping traditional practices. With materials advancements and ongoing research, 3D printing promises a future where personalized, precise, and patient-centric oral healthcare becomes the new norm.

Chapter 5: Teledentistry and Remote Consultations

Telehealth in Dentistry: The Evolution of Teledentistry

The realm of healthcare has undergone a transformative evolution with the integration of telehealth, a term that encompasses the use of digital communication technologies to provide remote medical services and consultations. In dentistry, this phenomenon has given rise to teledentistry, a revolutionary approach that redefines the way oral healthcare is delivered, reaching beyond physical boundaries and enhancing access, convenience, and patient engagement. This chapter delves into the evolution of teledentistry, illuminating its origins, applications, benefits, and the transformative impact it has on the oral health landscape.

Defining Teledentistry

The chapter commences by providing a comprehensive definition of teledentistry. Rooted in telehealth, teledentistry leverages digital communication technologies to facilitate remote consultations, diagnostics, treatment planning, and patient education. This section traces the historical development of teledentistry, from its nascent stages to its current prominence.

Origins and Genesis of Teledentistry

Teledentistry traces its roots to the broader telehealth movement, which emerged in response to the digital age's advancement and the need for accessible healthcare services. This convergence of technology, healthcare, and patient-centricity gave birth to teledentistry, aiming to bridge geographical gaps, optimize resource utilization, and empower patients to take an active role in their oral health.

The Multifaceted Applications of Teledentistry

Teledentistry is a versatile tool with applications that touch various aspects of oral healthcare:

Remote Consultations: Patients can connect with dentists virtually, seeking guidance, advice, and second opinions without the need for in-person visits.

Emergency Triage: Teledentistry enables quick assessment and guidance for dental emergencies, potentially preventing unnecessary trips to the dental office.

Follow-Up Care: Dentists can conduct virtual follow-up appointments to monitor treatment progress and address concerns post-treatment.

Patient Education: Teledentistry empowers patients with information about oral hygiene, preventive measures, and treatment options.

Smile Design: Patients can engage in virtual smile design consultations to envision and discuss potential aesthetic improvements.

Telediagnosis: Dentists can analyze images and videos sent by patients to provide preliminary diagnoses and treatment recommendations.

Teledentistry in Underserved Areas

Teledentistry has the potential to bridge the oral healthcare gap in underserved and rural areas:

Access to Specialists: Patients in remote areas can consult with specialized dentists without the need to travel long distances.

Preventive Education: Teledentistry facilitates oral health education in communities with limited access to dental resources.

Elderly Care: Teledentistry benefits elderly patients who may face challenges traveling to dental appointments.

Technological Enablers

Digital Imaging: High-resolution images and videos provide dentists with a comprehensive view of patients' oral conditions.

Secure Platforms: HIPAA-compliant video conferencing and communication platforms ensure patient data security and privacy.

Smartphone Apps: Mobile apps enable patients to capture and share oral health images, fostering seamless communication.

Benefits and Future Prospects

Enhanced Access: Teledentistry breaks down geographical barriers, enabling patients to access dental expertise regardless of location.

Convenience: Patients can receive consultations from the comfort of their homes, reducing the need for travel and time off work.

Preventive Focus: Teledentistry empowers patients with knowledge and preventive measures to maintain optimal oral health.

Patient-Centric Care: Patients play an active role in their oral health journey, fostering engagement and proactive healthcare decisions.

Conclusion: A Connected Future for Oral Health

The evolution of teledentistry epitomizes the fusion of healthcare and technology. From remote consultations to enhanced access, this transformative approach redefines the patient-dentist relationship, extending oral healthcare's reach beyond clinic walls. As technology continues to evolve, teledentistry will remain a pivotal force in shaping the future of oral health, ensuring that oral care is as accessible, convenient, and patient-centric as possible.

Remote Consultations and Virtual Visits

One of the primary applications of teledentistry lies in remote consultations. This section delves into how dental professionals can connect with patients in real time through video conferencing and digital communication platforms. Remote consultations offer a means of addressing patients' concerns, providing preliminary assessments, and guiding them on the next steps to take.

Diagnostic Advancements

Teledentistry has enabled dental practitioners to remotely diagnose certain conditions through the evaluation of images and videos provided by patients. This chapter explores how high-

resolution photographs, radiographs, and intraoral scans can be shared electronically, enabling dentists to make informed decisions about treatment plans without requiring patients to be physically present.

Virtual Treatment Planning and Monitoring

The ability to conduct virtual treatment planning and monitoring has transformed the way dental care is delivered. Through teledentistry, dental professionals can create comprehensive treatment plans, complete with visual simulations of potential outcomes. Moreover, they can remotely monitor patients' progress, ensuring that treatment is on track and making necessary adjustments as required.

Challenges and Considerations in Teledentistry

While teledentistry holds the promise of transforming oral healthcare delivery, it is not without its challenges and considerations. As this innovative approach continues to gain traction, it is imperative to acknowledge and address the complexities that arise in the virtual realm. This chapter explores the multifaceted challenges and critical considerations that dentists, patients, and stakeholders must navigate to ensure the successful integration of teledentistry into the oral healthcare landscape.

Regulatory and Legal Landscape

Licensure and Jurisdiction: Teledentistry often involves crossing state or national borders, raising questions about licensure and jurisdiction for dental practitioners.

Privacy and Security: Compliance with health information privacy regulations (such as HIPAA in the United States) is paramount to protect patient data during virtual consultations.

Standard of Care: Ensuring that the quality of care delivered through teledentistry is consistent with traditional in-person care remains a concern.

Technological Hurdles

Access to Technology: Disparities in internet access and digital literacy may hinder patients' ability to engage effectively in teledentistry.

Reliability: Technical glitches, connectivity issues, and software compatibility can impact the smoothness of virtual consultations.

Data Integrity: Protecting the confidentiality and integrity of patient data and electronic health records is of utmost importance.

Diagnostic Limitations

Physical Examination: Teledentistry lacks the ability to physically examine the patient, which can limit the accuracy of diagnoses.

Diagnostic Imaging: While high-quality images can be shared, a lack of tactile information may hinder the dentist's ability to make accurate diagnoses.

Treatment Planning: Complex treatment plans may be challenging to devise solely based on virtual interactions.

Patient and Provider Communication

Miscommunication: Non-verbal cues and physical interactions that contribute to effective communication in-person may be lost in virtual consultations.

Limited Engagement: Building patient rapport and trust can be more challenging in virtual interactions compared to face-to-face consultations.

Informed Consent: Clear communication of treatment options, risks, and benefits is crucial in teledentistry to ensure informed patient consent.

Reimbursement and Insurance

Reimbursement Models: Establishing appropriate reimbursement structures for teledentistry services remains an evolving challenge.

Insurance Coverage: Determining which teledentistry services are covered by insurance and the extent of coverage can be complex.

Patient Engagement and Follow-Up

Patient Compliance: Ensuring that patients follow through with treatment plans and post-consultation instructions may be more challenging in a virtual setting.

Emergency Situations: Determining how to handle dental emergencies that arise during virtual consultations requires clear protocols.

Cultural and Ethical Considerations

Cultural Competency: Dentists must navigate cultural differences in communication styles and healthcare expectations during virtual interactions.

Ethical Dilemmas: Ethical considerations, such as the appropriate use of teledentistry for certain diagnoses or the potential for overdiagnosis, need careful navigation.

Conclusion: Navigating the Virtual Terrain with Care

As teledentistry continues to redefine oral healthcare delivery, it is essential to approach its implementation with a comprehensive understanding of the challenges and considerations it poses. Mitigating these complexities requires collaboration between dental professionals, regulatory bodies, technology providers, and patients. Through ongoing education, adaptability, and an unwavering commitment to patient welfare, the dentistry community can harness the potential of teledentistry while addressing the ethical, legal, and technological intricacies that accompany this virtual revolution.

Conclusion

Chapter 5 unravels the potential of teledentistry to transcend geographical barriers and transform the way dental care is delivered. From remote consultations and diagnostics to virtual treatment planning and patient education, teledentistry brings convenience, accessibility, and efficiency to oral healthcare. As the technology continues to evolve and regulations adapt, teledentistry is poised to play an integral role in shaping the future of dental practice.

Chapter 6: Artificial Intelligence in Dentistry

AI-Assisted Diagnosis and Treatment Planning

In the dynamic landscape of modern dentistry, the integration of artificial intelligence (AI) has ushered in a transformative era, redefining the way diagnoses are made and treatment plans are formulated. This chapter delves into the realm of AI-assisted diagnosis and treatment planning, exploring how cutting-edge technologies harness the power of AI to enhance diagnostic accuracy, streamline treatment planning processes, and elevate patient care to unprecedented levels of precision.

The Marriage of AI and Dentistry

Artificial intelligence encompasses algorithms and technologies that enable machines to mimic human cognitive functions, such as learning, reasoning, and problem-solving. In dentistry, AI algorithms analyze vast amounts of data, from patient records to radiographic images, to support dentists in making well-informed clinical decisions.

Advancing Diagnosis with AI

Image Analysis: AI algorithms excel in analyzing dental radiographs, identifying subtle anomalies and patterns that might escape the human eye.

Early Detection: AI can aid in the early detection of conditions such as dental caries, periodontal disease, and even oral cancers, contributing to better patient outcomes.

Risk Assessment: AI tools assess patient data to predict the likelihood of future dental issues, allowing for proactive preventive strategies.

Personalized Treatment Planning

Data-Driven Insights: AI assimilates patient data, medical history, and treatment outcomes to offer personalized treatment options.

Treatment Simulation: AI enables dentists to simulate treatment outcomes, allowing patients to visualize the results of various interventions.

Predictive Modeling: AI algorithms predict treatment success rates based on patient characteristics, assisting dentists in selecting the most effective approaches.

Enhancing Clinical Efficiency

Workflow Optimization: AI streamlines administrative tasks, appointment scheduling, and patient communication, allowing dentists to focus more on patient care.

Quick Decision Support: AI provides instant access to evidence-based guidelines and literature, supporting clinical decision-making.

Patient Engagement: AI-powered educational tools enhance patient understanding, enabling them to actively participate in treatment decisions.

Challenges and Considerations

Data Privacy: Ensuring patient data security and compliance with regulations, such as HIPAA, is crucial in AI-powered dentistry.

Bias and Interpretation: AI algorithms may inadvertently inherit biases present in the training data, necessitating ongoing refinement.

Clinical Expertise: AI serves as a tool to aid clinical judgment, but human expertise remains paramount in interpreting AI-generated insights.

Human-AI Collaboration

Synergy: The optimal use of AI is as a supportive tool, enhancing dentists' abilities rather than replacing them.

Continual Learning: Dentists must stay informed about AI advancements to effectively integrate them into practice.

Ethical Considerations: Clear guidelines are needed for handling AI-generated diagnoses, especially in complex cases.

The Road Ahead: Pioneering Precision

AI-assisted diagnosis and treatment planning epitomize the fusion of human ingenuity and technological prowess. The integration of AI empowers dental professionals with data-driven insights, refined diagnostic capabilities, and streamlined treatment planning, ensuring a level of precision that was once beyond reach. As AI continues to evolve, dentistry stands at the threshold of a new era, one where patient care is enhanced, outcomes are optimized, and the journey towards precision-driven dentistry reaches new heights.

The integration of artificial intelligence (AI) into dentistry has ushered in a new era of data-driven decision-making, precision diagnostics, and personalized treatment planning. This

chapter delves into the profound impact of AI on the dental landscape, highlighting how machine learning algorithms are transforming the way oral healthcare is delivered.

Understanding Artificial Intelligence in Dentistry The chapter begins by elucidating the fundamental concepts of artificial intelligence and its applications in dentistry. AI involves the use of algorithms to analyze vast datasets, extract patterns, and make predictions based on historical information. This section outlines the potential of AI to augment clinical expertise, enhance diagnostics, and optimize treatment outcomes.

AI-Powered Diagnostics The transformative power of AI is evident in its ability to analyze dental images and identify subtle patterns that may elude the human eye. This section explores how AI algorithms can aid in the early detection of conditions such as cavities, periodontal disease, and oral cancers. By sifting through vast amounts of data, AI systems provide clinicians with more accurate and timely diagnostic insights.

Treatment Planning and Prediction AI-driven treatment planning is revolutionizing the way dental procedures are approached. Machine learning models can analyze patient data, historical treatment outcomes, and clinical guidelines to suggest personalized treatment plans. This section delves into how AI assists clinicians in selecting the most effective treatment options while factoring in individual patient factors.

Machine Learning in Oral Health Analysis

In the realm of oral health analysis, the integration of machine learning has ignited a paradigm shift, transforming the way data is harnessed to uncover invaluable insights. This chapter delves into the world of machine learning in oral health analysis, exploring how advanced algorithms leverage data to predict diseases, personalize treatment plans, and optimize patient care, marking a pivotal advancement towards precision-driven dentistry.

The Essence of Machine Learning

Machine learning is a subset of artificial intelligence that empowers computers to learn from data without being explicitly programmed. In oral health analysis, machine learning algorithms process vast amounts of clinical, radiographic, and patient data to recognize patterns, make predictions, and support evidence-based decision-making.

Enhancing Diagnosis and Predictive Analytics

Disease Prediction: Machine learning algorithms analyze patient data to predict the likelihood of developing oral diseases, such as periodontal disease or caries.

Risk Assessment: By integrating various patient characteristics and behaviors, machine learning identifies individuals at higher risk of dental issues, enabling proactive interventions.

Image Analysis: Algorithms decipher complex radiographic images, aiding in the early detection of anomalies and irregularities.

Tailored Treatment Planning

Personalized Treatment Plans: Machine learning tailors treatment plans based on individual patient data, optimizing outcomes and minimizing trial and error.

Treatment Response Prediction: Algorithms analyze patient responses to previous treatments, informing dentists about the most effective interventions for similar cases.

Optimizing Patient Care

Patient Engagement: Machine learning enhances patient engagement through personalized education, encouraging proactive oral health practices.

Treatment Recommendations: Algorithms assist dentists in recommending evidence-based treatment options, elevating the quality of care.

Workflow Efficiency: Machine learning streamlines administrative tasks, allowing dental professionals to allocate more time to patient care.

Challenges and Considerations

Data Quality: Accurate and comprehensive data are essential for machine learning algorithms to yield reliable insights.

Interpretability: Complex machine learning models can be challenging to interpret, requiring clear communication of results to clinical practitioners.

Bias Mitigation: Ensuring that algorithms do not perpetuate biases present in training data is a critical ethical consideration.

Human-Machine Synergy

Clinical Expertise: Machine learning serves as a powerful tool for dentists, supporting clinical judgment rather than replacing it.

Ongoing Learning: Dental professionals must continually educate themselves about machine learning advancements to optimally utilize this technology.

Ethical Boundaries: Clear guidelines are necessary to determine the extent of reliance on machine learning in patient diagnosis and treatment.

The Future: Empowering Precision in Oral Health

Machine learning in oral health analysis stands as a testament to the synergy between technology and healthcare. The integration of machine learning augments dental professionals' diagnostic prowess, offering personalized treatment strategies, and elevating patient care to levels of precision that were once unthinkable. As machine learning continues to evolve, the future of oral health analysis holds exciting prospects, promising a landscape where data-driven insights shape treatment paradigms and empower dental professionals to achieve unparalleled clinical outcomes.

The Role of Machine Learning The chapter shifts its focus to the role of machine learning in oral health analysis. By training algorithms on diverse datasets, machine learning models can predict disease progression, identify risk factors, and guide preventive measures. This section explores how machine learning enhances risk assessment and empowers patients to take proactive steps toward maintaining oral health.

Early Detection and Intervention Machine learning algorithms excel in early disease detection. The chapter delves into how these algorithms can analyze patient data, such as clinical records and imaging, to predict the likelihood of developing conditions like periodontal disease and dental caries. By enabling early intervention, machine learning contributes to better outcomes and reduced treatment costs.

Personalized Treatment Recommendations The potential of machine learning to provide personalized treatment recommendations is a cornerstone of patient-centric care. This section discusses how machine learning algorithms consider patient-specific factors, such as genetics, lifestyle, and oral microbiome, to create tailored treatment plans that optimize efficacy and patient satisfaction.

Challenges and Ethical Considerations

Data Privacy and Security As AI and machine learning rely heavily on patient data, ensuring data privacy and security is paramount. The chapter examines the challenges associated with data protection and the steps that dental practices must take to safeguard patient information in the digital age.

Ethical Considerations in AI-Driven Dentistry The integration of AI also raises ethical questions. This section explores topics such as transparency in AI algorithms, patient consent for AI-driven diagnostics and treatments, and the role of human oversight in decisions made by AI systems.

Conclusion

Chapter 6 unveils the transformative potential of artificial intelligence and machine learning in the field of dentistry. By harnessing the power of data analytics, AI-driven diagnostics, and

personalized treatment planning, dental professionals can enhance patient care and outcomes. While challenges and ethical considerations persist, the promise of AI in dentistry continues to grow, shaping the future of oral healthcare.

Chapter 7: Robotics and Automationin Dental Procedures

Robotic-Assisted Surgeries and Beyond

In the realm of modern dentistry, the convergence of robotics and healthcare has led to a remarkable transformation, reshaping the way surgical procedures are conducted and redefining the boundaries of precision. This chapter delves into the world of robotic-assisted surgeries in dentistry, shedding light on how these advanced technologies have elevated surgical outcomes, expanded treatment possibilities, and heralded a new era of patient-centric care.

The Dawn of Robotic-Assisted Surgeries

Robotic-assisted surgeries represent a marriage of engineering, technology, and medical expertise. These systems enable dental professionals to conduct procedures with enhanced precision, control, and visualization, ultimately translating to improved patient care.

Precision in Surgery

Enhanced Visualization: Robotic systems provide magnified, high-definition 3D visualizations of the surgical field, enabling dentists to operate with unparalleled precision.

Steady Instrumentation: Robotic arms eliminate human hand tremors, ensuring steady and controlled movements during intricate procedures.

Minimally Invasive Approaches: Robotic systems facilitate minimally invasive surgeries, leading to smaller incisions, reduced trauma, and faster recovery.

Expanding Treatment Horizons

Implant Placement: Robotic systems enable precise implant placement, optimizing stability and long-term success.

Oral and Maxillofacial Procedures: From tumor removal to reconstructive surgeries, robotics enable intricate procedures with greater precision.

Complex Cases: Robotic assistance proves invaluable in complex cases where traditional methods may pose challenges.

Patient-Centric Advantages

Reduced Complications: Enhanced precision minimizes the risk of complications, reducing postoperative pain and recovery time.

Customized Treatment: Robotics enable treatment plans tailored to each patient's unique anatomy and needs.

Patient Comfort: Minimally invasive approaches and faster recovery contribute to improved patient comfort.

Challenges and Future Prospects

Training and Familiarization: Dental professionals require specialized training to effectively operate robotic systems.

Cost: The initial investment and maintenance costs of robotic systems may pose financial challenges for dental practices.

Human-Robot Collaboration

Clinical Judgment: While robotics enhance precision, clinical expertise remains vital in decision-making during surgeries.

Surgical Innovation: As robotic technology advances, the range of procedures that can benefit from its precision is likely to expand.

Ethical and Regulatory Considerations

Informed Consent: Patients must be informed about the use of robotic systems in their treatment plans.

Regulatory Compliance: Robotic-assisted surgeries must adhere to existing healthcare regulations and standards.

Conclusion: Precision Redefined, Outcomes Elevated

Robotic-assisted surgeries in dentistry epitomize the convergence of innovation and healthcare. These advanced technologies empower dental professionals with unprecedented precision, enabling them to conduct intricate procedures with enhanced visualization and control. As robotic systems continue to evolve, the path ahead holds exciting prospects, promising a future where patient-centric care, innovation, and precision harmonize to elevate surgical outcomes, redefine treatment possibilities, and reshape the landscape of modern dentistry.

The integration of robotics and automation into dentistry has ushered in a new era of precision, efficiency, and enhanced patient outcomes. This chapter delves into the expanding role of robotics in dental procedures, from surgical interventions to routine tasks, showcasing how automation is transforming the practice of oral healthcare.

Robotic-Assisted Surgical Precision The chapter commences by elucidating the significance of robotic-assisted surgeries in dentistry. Robotic systems offer unparalleled precision, allowing dental professionals to perform complex procedures with submillimeter accuracy. This section explores how robotic arms, equipped with advanced sensors and imaging technologies, enhance surgical planning and execution.

Implant Placement and Oral Surgery Robotic systems have found a crucial role in implant dentistry and oral surgery. This section delves into how robotic arms can assist in accurate implant placement by precisely drilling implant sites, ensuring optimal positioning for long-term success. Additionally, robotic systems can aid in procedures such as bone grafting and soft tissue surgeries, minimizing invasiveness and postoperative complications.

Endodontics and Canal Preparation The chapter extends its exploration of robotics to endodontics. Robotic systems can navigate complex root canal systems with high precision,

ensuring thorough cleaning and shaping. This section discusses how automation improves the outcomes of root canal procedures by reducing the risk of procedural errors.

Automation in Prosthodontics and Orthodontics

The integration of automation technology has ushered in a transformative era in the fields of prosthodontics and orthodontics, redefining the way treatments are planned, executed, and delivered. This chapter delves into the world of automation in prosthodontics and orthodontics, illuminating how cutting-edge technologies have elevated precision, efficiency, and patient-centric care, ultimately shaping the future of restorative and corrective dentistry.

The Evolution of Automation

Automation technology encompasses a range of tools and systems that streamline and enhance various aspects of dental care, from treatment planning to fabrication and beyond. This technological integration revolutionizes traditional approaches, unlocking new possibilities for dental professionals and patients alike.

Automation in Prosthodontics

Digital Impressions: Automated intraoral scanners replace traditional molds, capturing precise digital impressions that enhance accuracy and patient comfort.

CAD/CAM Restorations: Computer-aided design (CAD) and computer-aided manufacturing (CAM) systems automate the fabrication of dental restorations, ensuring consistent quality.

Virtual Mock-ups: Digital tools enable dentists to create virtual mock-ups of restorations, allowing patients to preview their new smiles before treatment.

Automation in Orthodontics

Customized Appliances: Automation allows for the design and fabrication of personalized orthodontic appliances, ensuring optimal fit and comfort.

Treatment Planning: Automated software assists orthodontists in planning treatment trajectories and predicting tooth movement.

3D Printing: Automation technology facilitates the creation of orthodontic models, aligners, and other devices through additive manufacturing.

Advantages of Automation

Precision: Automation eliminates human errors and inconsistencies, resulting in precise treatment outcomes.

Efficiency: Automated workflows streamline processes, reducing treatment time and increasing patient turnover.

Patient Engagement: Visualization tools enabled by automation empower patients to actively participate in their treatment decisions.

Customization: Automation allows for personalized treatment plans and restorations tailored to each patient's unique needs.

Challenges and Considerations

Initial Investment: The adoption of automation technology requires upfront investment in equipment and training.

Learning Curve: Dental professionals need to familiarize themselves with new software and tools to effectively leverage automation.

Ethical Use: While automation enhances efficiency, clinical judgment remains essential in treatment planning and decision-making.

Human-Machine Synergy

Clinical Expertise: Automation serves as a tool to enhance clinical judgment rather than replace it.

Continual Learning: Dental professionals must stay updated with automation advancements to harness their full potential.

Patient-Centric Care

Patient Experience: Automated workflows minimize chair time, enhancing patient comfort and convenience.

Treatment Outcomes: Precision-driven treatments achieved through automation lead to improved clinical outcomes and patient satisfaction.

Conclusion: Precision and Innovation Unleashed

Automation in prosthodontics and orthodontics epitomizes the union of technology and dental care. By enhancing precision, streamlining workflows, and offering personalized treatment solutions, automation technology transforms traditional practices and elevates patient experiences. As automation continues to evolve, these fields stand at the brink of a new era, one where the marriage of innovation and clinical expertise shapes the future of

restorative and corrective dentistry, ensuring treatments that are efficient, precise, and patient-centric.

Digital Fabrication and Automation Automation has revolutionized prosthodontics and orthodontics by expediting the fabrication of dental devices. This section delves into how computer-aided design (CAD) and computer-aided manufacturing (CAM) systems, coupled with robotics, streamline the production of crowns, bridges, dentures, and orthodontic appliances. Automated milling and 3D printing systems enable the creation of highly accurate and customized devices.

Orthodontic Aligner Manufacturing Robotic automation has also transformed the manufacturing of orthodontic aligners. This section explores how robotics can precisely trim and shape aligners, ensuring a snug fit and comfortable patient experience. Automation in aligner fabrication enhances treatment efficiency while maintaining treatment accuracy.

Challenges and Future Directions

Integration and Training While the benefits of robotics and automation are undeniable, their successful integration into dental practice requires training and adaptation. This section discusses the challenges associated with learning to operate robotic systems and integrating them into existing workflows.

Patient Acceptance and Ethics The chapter delves into patient acceptance of robotic-assisted procedures. Addressing patient concerns and communicating the benefits of robotics in improving procedural outcomes is crucial. Additionally, ethical considerations related to robotic autonomy, patient consent, and the role of human supervision are explored.

Conclusion

Chapter 7 unveils the transformative potential of robotics and automation in the realm of dental procedures. From precision-guided surgeries to automated prosthodontics and orthodontics, robotics is reshaping the landscape of oral healthcare. As dental professionals continue to harness the power of automation, the future promises not only improved procedural outcomes but also expanded capabilities that elevate the standard of care.

Chapter 8: Nanotechnology Applications in Dentistry

Nanomaterials and Their Impact on Oral Healthcare

In the realm of oral healthcare, the integration of nanomaterials has heralded a ground-breaking revolution, unlocking new dimensions of precision, efficacy, and innovation. This chapter delves into the world of nanomaterials and their profound impact on oral healthcare, illuminating how these tiny structures have paved the way for advanced therapies, enhanced diagnostics, and transformative treatment approaches, ultimately redefining the landscape of dental care.

Understanding Nanotechnology in Dentistry The chapter commences by demystifying the fundamentals of nanotechnology and its integration into oral healthcare. Nanotechnology involves the manipulation of materials at the nanoscale, where properties and behaviors differ from those at larger scales. This section lays the foundation for exploring how nanotechnology is revolutionizing dentistry.

Unveiling the Nanoscale

Nanomaterials are materials with structures and properties engineered at the nanoscale—measured in nanometers. Their unique properties arise from their minuscule size, granting them exceptional reactivity, surface area, and interactions with biological systems.

Advancing Diagnostics and Imaging

Nanoparticles for Imaging: Nanoparticles can be engineered to carry contrast agents, enhancing the visualization of oral structures in imaging techniques like X-rays and MRI.

Early Disease Detection: Nanoscale sensors can detect biomarkers of oral diseases at their earliest stages, enabling proactive interventions.

Precision Drug Delivery

Targeted Therapies: Nanoparticles enable precise delivery of therapeutic agents to specific oral sites, minimizing side effects and enhancing efficacy.

Oral Cancer Treatment: Nanoparticles can transport chemotherapeutic agents directly to cancer cells, reducing damage to healthy tissues.

Enhancing Restorative Dentistry

Nanocomposites: Dental restorations made from nanocomposites possess improved strength, durability, and aesthetics.

Bioactive Fillings: Nanomaterials infused with remineralizing agents can aid in repairing tooth structure damaged by caries.

Antimicrobial Effects

Nanoparticle Coatings: Dental implants and orthodontic appliances coated with antimicrobial nanoparticles mitigate the risk of infections.

Silver Nanoparticles: Silver nanoparticles exhibit potent antimicrobial properties, preventing the growth of harmful oral bacteria.

Challenges and Opportunities

Biocompatibility: Ensuring the safety and biocompatibility of nanomaterials in oral tissues is crucial.

Regulatory Hurdles: Nanomaterials' novel properties may pose challenges in aligning with existing dental material regulations.

Ethical Considerations

Long-Term Effects: The long-term effects of nanomaterial exposure in oral healthcare require thorough investigation.

Environmental Impact: Proper disposal and management of nanomaterials to minimize their environmental impact are ethical considerations.

Human-Material Interaction

Clinical Expertise: While nanomaterials offer transformative potential, clinical judgment remains essential in treatment decisions.

Continual Learning: Dental professionals must stay updated on nanomaterial advancements to leverage their full potential.

The Dawn of Nanodentistry

Nanomaterials' integration into oral healthcare epitomizes the confluence of science, medicine, and innovation. From targeted therapies to enhanced diagnostics, nanomaterials have unlocked a realm of possibilities that redefine the boundaries of dental care. As nanotechnology continues to evolve, the future of oral healthcare holds exciting prospects, promising a landscape where treatments are precise, therapies are tailored, and patient

outcomes are optimized—ushering in a new era of dentistry enriched by the power of nanotechnology.

Nanomaterials in Restorative Dentistry One of the primary applications of nanotechnology lies in restorative dentistry. This section delves into how nanomaterials, such as nanoparticles and nanocomposites, are incorporated into dental materials. Nanoparticles enhance the properties of materials like resin composites, making them stronger, more durable, and resistant to wear. This nanoscale reinforcement translates into longer-lasting restorations with improved aesthetics.

Anti-Microbial Nanoparticles The chapter shifts its focus to the role of nanoparticles in combating oral infections. Nanoscale antimicrobial agents have demonstrated the ability to inhibit bacterial growth and biofilm formation. This section explores how these nanoparticles can be integrated into dental materials, such as toothpaste and mouth rinses, to provide sustained protection against oral pathogens.

Nanotechnology-Enabled Drug Delivery

In the landscape of modern medicine, nanotechnology has emerged as a game-changer, offering innovative solutions to challenges in drug delivery. This chapter delves into the realm of nanotechnology-enabled drug delivery in oral healthcare, shedding light on how

nanoscale structures are harnessed to revolutionize therapeutic interventions, enhance treatment outcomes, and usher in a new era of precision medicine tailored to individual patient needs.

Unveiling Nanotechnology in Drug Delivery

Nanotechnology involves manipulating materials at the nanoscale—often on the order of billionths of a meter—to engineer structures with unique properties. In oral healthcare, nanotechnology has paved the way for novel drug delivery systems that offer unparalleled precision and effectiveness.

Enhanced Drug Bioavailability

Nanoformulations: Drugs encapsulated in nanoparticles or nanocarriers exhibit improved solubility and stability, increasing their bioavailability.

Targeted Delivery: Nanocarriers can be designed to release drugs at specific oral sites, reducing systemic side effects.

Local Therapies: Nanoparticles can be loaded with therapeutic agents to provide sustained local treatment, as seen in periodontal diseases.

Personalized Treatments

Tailored Nanocarriers: Nanotechnology allows for customization of drug carriers to match patient characteristics and treatment needs.

Theranostic Platforms: Nanoparticles can carry both therapeutic agents and diagnostic agents, enabling simultaneous treatment and monitoring.

Challenges and Considerations

Biocompatibility: Ensuring the compatibility of nanomaterials with oral tissues is paramount for patient safety.

Regulatory Complexities: The unique properties of nanoscale materials may necessitate novel regulatory approaches.

Ethical and Safety Considerations

Long-Term Effects: Long-term exposure to nanomaterials in oral tissues requires thorough investigation.

Environmental Impact: Proper disposal and management of nanomaterials are ethical concerns.

Human-Nanomaterial Interaction

Clinical Expertise: While nanotechnology offers transformative potential, clinical judgment remains essential in treatment decisions.

Continual Learning: Dental professionals must stay updated on nanotechnology advancements to harness their full potential.

Precision Redefined, Outcomes Elevated

Nanotechnology-enabled drug delivery in oral healthcare marks the convergence of scientific innovation and medical progress. By facilitating targeted therapies, enhancing drug bioavailability, and enabling personalized treatments, nanotechnology empowers dental professionals to elevate patient care to unprecedented levels of precision. As nanotechnology continues to advance, the future holds exciting prospects, promising a landscape where therapeutic interventions are tailored, treatments are effective, and oral healthcare reaches new heights of therapeutic excellence.

Nanoparticles as Drug Carriers Nanotechnology has enabled precise and targeted drug delivery in dentistry. This section delves into how nanoparticles can serve as carriers for therapeutic agents, allowing controlled release directly to affected areas. This approach

minimizes systemic side effects and enhances the efficacy of treatments for conditions such as periodontal disease and oral cancers.

Local Anesthetic Delivery Systems Nanotechnology has also revolutionized local anesthesia administration. This section discusses how nanogels and nanospheres can encapsulate anesthetics, enabling slow and controlled release. This innovative approach prolongs the duration of anesthesia, reduces the need for multiple injections, and enhances patient comfort during dental procedures.

Tissue Engineering and Regenerative Dentistry

Biocompatible Scaffolds The potential of nanotechnology in tissue engineering is profound. This section explores how nanomaterials can be integrated into biocompatible scaffolds, facilitating the regeneration of dental tissues. Nanoscale features of these scaffolds mimic the natural extracellular matrix, promoting cell adhesion, growth, and tissue regeneration.

Pulp Regeneration and Dentin Repair Nanotechnology-driven advances in regenerative dentistry offer hope for preserving dental pulp and repairing damaged dentin. This section discusses how nanomaterials can stimulate stem cell differentiation and promote dentin remineralization. The integration of nanofibers, nanoparticles, and growth factors contributes to the regeneration of functional dental tissues.

Challenges and Ethical Considerations

Risk Assessment and Biocompatibility While nanotechnology holds immense promise, evaluating the safety and biocompatibility of nanomaterials is imperative. This section delves into the challenges of assessing potential risks associated with nanoparticle exposure and ensuring that nanomaterials used in dentistry do not compromise patient health.

Ethical Considerations in Nanodentistry The chapter concludes by addressing ethical considerations related to nanodentistry. Topics such as informed consent, transparency about nanoparticle use, and potential long-term effects are discussed. Striking a balance between harnessing the benefits of nanotechnology and safeguarding patient welfare is pivotal.

Conclusion

Chapter 8 unveils the transformative potential of nanotechnology applications in dentistry. From enhancing restorative materials to enabling targeted drug delivery and tissue regeneration, nanotechnology is revolutionizing oral healthcare. While challenges and ethical considerations persist, the promise of nanodentistry continues to grow, ushering in a future where precision, regeneration, and patient-centric care intersect.

Chapter 9: Virtual Reality (VR) and Augmented Reality (AR) in Dental Education

Immersive Learning and Enhanced Pedagogy

The integration of virtual reality (VR) and augmented reality (AR) technologies into dental education has ushered in a transformative era of immersive learning and enhanced pedagogy. This chapter delves into the dynamic landscape of VR and AR applications in dental education, showcasing how these technologies are reshaping the way future dental professionals learn, practice, and refine their skills.

The realm of dental education is undergoing a remarkable transformation, thanks to the integration of immersive learning technologies that enhance pedagogical approaches. This chapter delves into the world of immersive learning and its impact on dental education, illuminating how cutting-edge technologies are revolutionizing teaching methodologies, elevating student engagement, and preparing the next generation of dental professionals for the challenges of the modern healthcare landscape.

The Advent of Immersive Learning

Immersive learning harnesses technology to create simulated environments that immerse learners in realistic scenarios, fostering experiential learning, critical thinking, and active participation.

Virtual Reality (VR): VR technology creates immersive 3D environments that students can explore and interact with using specialized headsets and controllers.

Augmented Reality (AR): AR overlays digital content onto the real world, enhancing learners' understanding of complex subjects through interactive visualizations.

Benefits for Dental Education

Enhanced Engagement: Immersive learning captivates students' attention and fosters active participation in their learning journeys.

Realistic Simulations: Simulated patient encounters and procedures in immersive environments provide safe practice opportunities for students.

Critical Thinking: Immersive scenarios challenge students to think critically and make informed decisions in realistic contexts.

Empowered Exploration: VR and AR allow students to explore anatomical structures and dental procedures in intricate detail.

Clinical Preparation: Immersive learning helps students develop clinical reasoning and confidence before engaging with real patients.

Challenges and Considerations

Technology Integration: Implementing immersive learning requires investment in hardware, software, and training.

Content Development: Creating high-quality immersive content demands resources, time, and expertise.

Adaptive Learning: Ensuring that the immersive learning experience caters to diverse learning styles is crucial.

Ethical and Pedagogical Considerations

Patient Privacy: Immersive scenarios involving patient data must prioritize patient privacy and confidentiality.

Effective Integration: Immersive learning should complement, not replace, traditional teaching methods to ensure comprehensive education.

Human-Technology Balance

Educator Role: While technology enhances learning, educators remain vital in guiding discussions and providing context.

Continuous Learning: Educators must adapt to new technologies and pedagogical approaches to maximize their benefits.

Navigating a New Educational Frontier

Immersive learning's integration into dental education epitomizes the evolution of teaching in the digital age. By blending technology with pedagogical principles, educators can engage students, cultivate critical thinking, and bridge the gap between theoretical knowledge and practical application. As immersive learning technologies continue to evolve, the future of dental education holds exciting prospects, promising a landscape where students are not just passive learners but active participants in their educational journey—equipped with the skills, confidence, and insights needed to excel in the dynamic field of dentistry.

Understanding VR and AR in Dental Education The chapter commences by elucidating the distinctions between virtual reality and augmented reality. VR immerses users in entirely simulated environments, while AR overlays digital content onto the real world. This section provides a foundational understanding of how these technologies are harnessed to create interactive and engaging educational experiences.

Virtual Simulation of Dental Procedures One of the primary applications of VR in dental education lies in simulating dental procedures. This section explores how students can practice procedures such as tooth extractions, cavity preparations, and crown placements in a realistic virtual environment. VR simulations allow students to refine their hand-eye coordination, spatial awareness, and decision-making skills before entering clinical practice. AR-Enhanced Learning Environments The chapter shifts its focus to augmented reality's role in dental education. AR enriches the learning environment by overlaying digital information onto physical objects. This section discusses how AR can provide students with real-time guidance during hands-on exercises, allowing them to visualize anatomical structures and proper techniques while performing tasks.

Dental Anatomy and 3D Visualization

Virtual Dissection and Exploration VR and AR technologies offer novel ways to teach dental anatomy and enhance 3D visualization skills. Students can virtually dissect anatomical

structures, explore dental morphology, and gain an in-depth understanding of complex spatial relationships. This section delves into how these technologies bridge the gap between theoretical knowledge and practical application.

Case-Based Learning with Interactive Models Dental education is enriched through case-based learning, and VR/AR technologies amplify this approach. This section explores how students can interact with digital 3D models of patient cases, navigating through complex scenarios and making decisions that impact treatment outcomes. This immersive experience prepares students for the complexities of clinical practice.

Challenges and Future Directions

Technological Integration and Accessibility While VR and AR offer transformative learning experiences, their successful integration into dental curricula requires access to suitable hardware and software. This section discusses the challenges associated with ensuring that educational institutions can provide the necessary resources for widespread adoption.

Ethical Considerations and Curriculum Design The chapter addresses ethical considerations in using VR and AR technologies for dental education. It explores topics such as patient privacy, data security, and ensuring that virtual simulations align with ethical standards.

Additionally, the chapter discusses the need for thoughtful curriculum design that maximizes the benefits of these technologies.

Conclusion

Chapter 9 unveils the potential of virtual reality and augmented reality in reshaping dental education. From immersive procedural simulations to interactive anatomy learning, these technologies elevate the educational experience by fostering engagement, skill development, and deep understanding. As dental education continues to evolve, VR and AR stand as powerful tools that empower future dental professionals to embark on their clinical journeys with confidence and proficiency.

Chapter 10: Big Data and Analyticsin Oral Healthcare

Harnessing Data for Informed Decision-Making

The era of big data and analytics has revolutionized numerous industries, and oral healthcare is no exception. This chapter delves into the transformative role of big data and analytics in oral healthcare, showcasing how data-driven insights are shaping clinical practices, patient outcomes, and the future of dental care.

In the age of digital transformation, the dental field is increasingly leveraging data analytics to make informed decisions that drive practice growth, enhance patient care, and optimize operational efficiency. This chapter delves into the world of data analytics in dentistry, illuminating how the strategic use of data empowers dental professionals to uncover insights, predict trends, and shape the future of oral healthcare.

The Data Revolution in Dentistry

Data analytics involves extracting actionable insights from large volumes of data to drive evidence-based decision-making. In dentistry, the proliferation of electronic health records, patient data, and practice management information has paved the way for a data-driven approach to improving every facet of oral healthcare.

Strategic Insights

Patient Demographics: Data analytics reveal patient demographics, helping practices target specific populations and tailor services.

Treatment Outcomes: Analyzing treatment data enables practices to identify effective interventions and refine treatment protocols.

Operational Efficiency: Insights into appointment scheduling and workflow allow practices to optimize resource allocation.

Predictive Analytics

Patient Trends: Analyzing historical data can predict patient trends, helping practices adapt to seasonal variations in demand.

Supply Chain Management: Predictive analytics optimize inventory management, ensuring essential supplies are available when needed.

Patient Retention: Predictive modeling identifies patients at risk of attrition, allowing proactive retention strategies.

Enhancing Patient Care

Personalized Treatment: Analytics enable personalized treatment plans based on patient history, preferences, and clinical data.

Early Intervention: Monitoring trends in patient health data can trigger early intervention for conditions like periodontal disease.

Patient Engagement: Data-driven insights improve patient communication, encouraging active participation in their oral health.

Challenges and Considerations

Data Quality: Reliable and accurate data are essential for meaningful analysis and decision-making.

Privacy and Security: Ensuring patient data security and compliance with regulations is paramount.

Data Interpretation: Data analytics require expertise to interpret results and make actionable decisions.

Ethical and Regulatory Considerations

Informed Consent: Patients should be informed about data collection and its intended uses.

Regulatory Compliance: Data collection and analysis must adhere to healthcare regulations and privacy laws.

Human-Data Synergy

Clinical Expertise: Data analytics support clinical judgment but don't replace it.

Continuous Learning: Dental professionals must stay updated on data analytics advancements to harness their full potential.

A New Age of Informed Dentistry

Data analytics' integration into dentistry marks a transformative shift towards evidence-based, patient-centric care. By transforming raw data into actionable insights, dental professionals can optimize operations, enhance patient care, and predict emerging trends. As data analytics continue to evolve, the future of oral healthcare holds exciting prospects, promising a landscape where decisions are informed by data, precision is optimized, and patient experiences are elevated through the strategic use of information.

Understanding Big Data and Analytics in Oral Healthcare The chapter commences by demystifying the concepts of big data and analytics. Big data refers to the massive volumes of information generated in healthcare settings, including patient records, images, and treatment histories. Analytics involves the use of sophisticated algorithms to mine this data for valuable insights. This section provides a foundation for exploring the applications of these technologies in dentistry.

Clinical Decision Support Systems One of the primary applications of big data and analytics lies in clinical decision support systems. This section explores how algorithms can analyze patient data to provide clinicians with evidence-based recommendations for diagnoses, treatment plans, and interventions. By leveraging historical data and treatment outcomes, these systems enhance the precision and efficacy of clinical decision-making.

Predictive Analytics and Preventive Dentistry The chapter shifts its focus to predictive analytics in oral healthcare. By analyzing large datasets, predictive models can identify patterns and risk factors associated with oral diseases. This section delves into how these models can forecast the likelihood of conditions such as periodontal disease and dental caries, enabling proactive preventive measures.

Personalized Treatment Strategies The potential of big data and analytics to drive personalized treatment strategies is profound. This section discusses how patient data, including genetics, lifestyle, and treatment history, can be analyzed to create customized treatment plans. Tailoring treatments to individual patient profiles enhances outcomes and patient satisfaction.

Improving Practice Efficiency and Workflow

Operational Analytics for Practice Management Analytics also play a crucial role in optimizing practice efficiency. This section explores how operational data, such as appointment scheduling, patient flow, and resource allocation, can be analyzed to enhance workflow and reduce bottlenecks. By identifying areas of improvement, practices can streamline operations and enhance patient experiences.

Patient Engagement and Communication Big data and analytics contribute to patient engagement by tailoring communication strategies to individual preferences and needs. This section discusses how data-driven insights can inform the creation of targeted educational materials, reminders, and follow-up communications, fostering a stronger patient-provider relationship.

Challenges and Ethical Considerations

As digital dentistry continues to reshape the landscape of oral healthcare, it brings with it a host of challenges and ethical considerations that must be carefully navigated. This chapter delves into the multifaceted challenges and ethical dilemmas that arise in the realm of digital dentistry, shedding light on the complexities that dental professionals, patients, and stakeholders must address to ensure the responsible and beneficial implementation of advanced technologies.

Challenges in Digital Dentistry

Technological Integration: Implementing and adopting new technologies requires investment in training, equipment, and infrastructure.

Data Security: Protecting patient data from breaches and ensuring compliance with privacy regulations is paramount.

Standardization: The lack of uniform standards across digital platforms can hinder interoperability and data exchange.

Training and Education: Dental professionals need ongoing training to stay updated with evolving digital tools and techniques.

Patient Accessibility: Not all patients have equal access to advanced digital dental services, leading to disparities in care.

Ethical Considerations

Patient Autonomy: Balancing patient autonomy with technological recommendations requires clear communication and informed consent.

Informed Consent: Patients should be fully informed about the benefits, risks, and alternatives of digital procedures.

Privacy: Protecting patient data privacy is essential in an era of digital records and communication.

Overdiagnosis and Overtreatment: Advanced technologies can lead to unnecessary interventions if not used judiciously.

Accuracy and Reliability: Ensuring the accuracy and reliability of digital tools and diagnostic outputs is a moral imperative.

Transparency: Dental professionals should be transparent about the role of technology in diagnosis and treatment planning.

Safeguarding Professional Judgment

Clinical Expertise: Digital tools enhance clinical practice but should never replace the expertise of dental professionals.

Human Oversight: Dental professionals must exercise their judgment in interpreting digital outputs and making treatment decisions.

Continuous Learning: Keeping up with technology advancements is crucial to wield digital tools effectively and ethically.

Navigating the Digital Terrain with Ethics

As digital dentistry becomes increasingly integrated into oral healthcare, it is imperative to address challenges and ethical considerations head-on. Dental professionals must strike a balance between leveraging the benefits of advanced technologies and upholding ethical principles that prioritize patient welfare, autonomy, and privacy. By fostering a culture of transparency, informed decision-making, and continuous education, the dental community can embrace digital dentistry responsibly, ensuring that technology serves as a tool to enhance, rather than compromise, the ethical foundations of the profession.

Data Security and Privacy The chapter addresses the challenges associated with data security and patient privacy. As the collection and storage of sensitive patient information increase, it becomes imperative to implement robust data protection measures and adhere to regulatory guidelines.

Ethical Use of Data The ethical use of patient data is paramount. This section explores topics such as informed consent for data collection and sharing, transparency about how data is used, and the responsibility of dental professionals to ensure that patient information is handled ethically and responsibly.

Conclusion

Chapter 10 unveils the transformative potential of big data and analytics in oral healthcare. From clinical decision support systems to predictive analytics and personalized treatment strategies, these technologies empower dental professionals with data-driven insights that enhance patient care and practice efficiency. While challenges and ethical considerations persist, the promise of big data continues to grow, shaping the future of oral healthcare where informed decisions are driven by comprehensive insights.

Chapter 11: Digital Workflow Optimization

Streamlining Dental Workflows Through Digitalization

The digitization of dental workflows has ushered in a new era of efficiency, accuracy, and enhanced patient experiences. This chapter delves into the concept of digital workflow optimization, showcasing how the integration of digital technologies is transforming the way dental practices operate, collaborate, and provide comprehensive patient care.

In the dynamic landscape of modern dentistry, the integration of digital technologies has ushered in a new era of streamlined workflows, enhancing practice efficiency and elevating patient care. This chapter delves into the world of digitalization in dental workflows, illuminating how advanced technologies are revolutionizing administrative processes, clinical procedures, and patient interactions, ultimately shaping the future of dentistry through enhanced precision and optimal resource utilization.

The Digital Transformation in Dentistry

Digitalization involves the conversion of traditional processes into digital formats, harnessing technology to automate and optimize various aspects of dental practice management and patient care.

Administrative Efficiency

Electronic Health Records: Digital patient records streamline documentation, enable quick retrieval, and enhance organization.

Appointment Management: Online scheduling systems reduce manual booking efforts and enhance patient convenience.

Billing and Payments: Digital payment options and automated invoicing improve financial transactions and record keeping.

Clinical Advancements

Digital Imaging: Radiographs, intraoral scans, and 3D imaging facilitate precise diagnostics and treatment planning.

CAD/CAM Technology: Computer-aided design and manufacturing streamline the creation of restorations and appliances.

Treatment Simulation: Digital tools allow patients to visualize treatment outcomes and make informed decisions.

Patient Engagement

Telehealth: Digital platforms enable remote consultations, enhancing accessibility and patient-provider communication.

Educational Resources: Online materials empower patients with information to actively participate in their oral health management.

Treatment Communication: Digital platforms facilitate clear communication between dental professionals, specialists, and laboratories.

Efficiency Gains

Time Optimization: Digital workflows reduce time spent on administrative tasks, allowing more focus on patient care.

Resource Utilization: Automation and digitalization minimize wastage of materials and resources.

Data-Driven Insights: Digital tools provide valuable insights into practice performance, aiding strategic decision-making.

Challenges and Considerations

Technological Investment: Initial costs and training are associated with adopting and integrating digital tools.

Data Security: Safeguarding patient data from breaches and cyber threats is critical.

Change Management: Transitioning to digital workflows requires adapting to new processes and technologies.

Ethical and Patient-Centric Approach

Informed Consent: Patients should understand the implications of digital technologies in their treatment.

Data Privacy: Patient data must be protected in compliance with healthcare regulations.

Human-Digital Synergy

Clinical Judgment: Digital tools enhance clinical capabilities but do not replace the expertise of dental professionals.

Continuous Learning: Staying updated with digital advancements is essential to leverage their full potential.

Conclusion: A Digital Odyssey in Dentistry

Digitalization's integration into dental workflows marks a transformative journey towards efficiency, precision, and patient-centric care. By leveraging digital tools to optimize administrative tasks, enhance clinical procedures, and empower patient interactions, dental professionals are shaping a future where the art of dentistry seamlessly integrates with the power of technology. As digitalization continues to evolve, the horizon of dentistry holds exciting possibilities, promising a landscape where streamlined workflows pave the way for enhanced patient experiences, superior clinical outcomes, and a profession poised for excellence in the digital age.

Understanding Digital Workflow Optimization The chapter commences by elucidating the essence of digital workflow optimization. Digitalization involves the integration of various technologies, from digital impressions and treatment planning software to CAD/CAM

systems and electronic health records. This section lays the foundation for exploring how these technologies synergize to streamline workflows.

Digital Impressions and Scanning Technologies One of the primary components of digital workflow optimization lies in digital impressions and scanning technologies. This section explores how intraoral scanners replace traditional impression materials, capturing precise digital replicas of patients' oral structures. By eliminating the discomfort associated with physical impressions, digital impressions enhance patient satisfaction and expedite treatment planning.

Interdisciplinary Collaboration and Communication

Enhancing Interdisciplinary Communication Digital workflows facilitate seamless collaboration between dental professionals. This section discusses how digital records and treatment plans can be shared electronically, enhancing interdisciplinary communication and ensuring that all specialists involved in a patient's care have access to up-to-date information. Refining Treatment Planning and Case Presentation The chapter shifts its focus to how digital workflows optimize treatment planning and case presentation. Treatment plans can be visualized in 3D models, enabling patients to better comprehend proposed procedures. This interactive approach fosters informed decision-making and empowers patients to actively participate in their treatment journeys.

CAD/CAM Systems and Chairside Milling

CAD/CAM Integration The integration of computer-aided design (CAD) and computer-aided manufacturing (CAM) systems is a cornerstone of digital workflow optimization. This section explores how digital designs are converted into physical restorations through milling or 3D printing. Chairside milling allows for immediate fabrication of restorations, reducing turnaround times and enhancing patient convenience.

Precision and Customization Digital workflow optimization enhances the precision and customization of dental restorations. CAD software enables dental professionals to design restorations with meticulous accuracy, ensuring optimal fit and aesthetics. This section delves into how the digitization of the design and fabrication process enhances restorative outcomes.

Challenges and Integration Strategies

Technological Integration While digital workflow optimization offers numerous benefits, integrating multiple digital technologies can pose challenges. This section discusses strategies for selecting compatible systems, training staff, and ensuring smooth transitions during the integration process.

Data Management and Security The chapter addresses data management and security considerations. As practices handle increasing volumes of digital patient information, it

becomes crucial to implement robust data protection measures, adhere to privacy regulations, and safeguard against potential breaches.

Conclusion

Chapter 11 unveils the transformative potential of digital workflow optimization in dentistry. From digital impressions to CAD/CAM systems, these technologies streamline workflows, enhance interdisciplinary collaboration, and elevate patient experiences. While challenges related to integration and data security persist, the promise of optimized digital workflows continues to reshape the landscape of dental practice, forging a future where precision, efficiency, and patient-centered care intersect.

Chapter 12: Teleorthodontics and Remote Aligner Treatments

Revolutionizing Orthodontic Care Through Telehealth

The convergence of orthodontics and telehealth has given rise to teleorthodontics, a transformative approach that redefines how orthodontic treatments are delivered and monitored. This chapter delves into the world of remote aligner treatments, showcasing how teleorthodontics is reshaping orthodontic care through virtual consultations, remote monitoring, and innovative treatment modalities.

The field of orthodontics is undergoing a transformative revolution with the integration of telehealth, ushering in a new era of convenience, accessibility, and patient-centric care. This chapter delves into the world of telehealth in orthodontics, illuminating how virtual technologies are reshaping treatment paradigms, enhancing patient interactions, and redefining the path to beautifully aligned smiles.

Telehealth in Orthodontics: Redefining Boundaries

Telehealth involves the use of technology to provide remote healthcare services, eliminating geographical barriers and offering a virtual channel for patient-provider interactions.

Remote Consultations

Virtual Assessments: Patients can submit photos and videos to orthodontists for initial evaluations and treatment recommendations.

Treatment Planning: Orthodontists create customized treatment plans based on remote assessments and patient goals.

Continual Monitoring: Regular virtual check-ins enable orthodontists to monitor treatment progress and make adjustments.

Enhanced Patient Convenience

Time Savings: Virtual appointments eliminate the need for travel and waiting times, enhancing patient convenience.

Flexible Scheduling: Patients can engage with orthodontists without disrupting work, school, or daily routines.

Global Reach: Telehealth transcends borders, allowing patients to access specialist care regardless of location.

Precision and Personalization

Digital Impressions: Intraoral scans provide orthodontists with accurate digital models for treatment planning.

Treatment Simulation: Virtual tools allow patients to visualize their treatment progression and anticipated outcomes.

Data-Driven Decisions: Remote monitoring provides data insights that inform adjustments for optimal results.

Challenges and Considerations

Technological Accessibility: Patients need access to devices and internet connectivity for virtual interactions.

Data Security: Protecting patient data during virtual consultations and interactions is crucial.

Clinical Limitations: Complex cases or emergencies may require in-person evaluations and interventions.

Ethical and Regulatory Aspects

Informed Consent: Patients should understand the scope and limitations of virtual orthodontic care.

Regulatory Compliance: Telehealth services must adhere to healthcare regulations and guidelines.

Human-Technology Synergy

Clinical Judgment: Telehealth enhances patient care but doesn't replace the expertise of orthodontic professionals.

Continuous Learning: Staying updated with virtual tools and telehealth advancements is essential.

Conclusion: A New Dimension in Orthodontic Care

Telehealth's integration into orthodontics has transcended conventional boundaries, offering a virtual realm where patients and orthodontists connect seamlessly. By embracing virtual

consultations, treatment planning, and remote monitoring, orthodontic care becomes not only more accessible but also more patient-centric and personalized. As telehealth continues to evolve, the future of orthodontics holds exciting possibilities, promising a landscape where beautifully aligned smiles are achieved through the intersection of technology, expertise, and patient empowerment.

Understanding Teleorthodontics and Remote Aligner Treatments The chapter commences by demystifying the concept of teleorthodontics. Teleorthodontics involves the use of telehealth technologies to remotely assess, plan, and monitor orthodontic treatments. This section lays the foundation for exploring how remote aligner treatments are revolutionizing the orthodontic landscape.

Virtual Consultations and Treatment Planning One of the primary applications of teleorthodontics lies in virtual consultations and treatment planning. This section discusses how orthodontists can remotely connect with patients through video conferencing and digital communication platforms. Virtual consultations allow orthodontists to assess patients' needs, discuss treatment options, and create personalized treatment plans.

Remote Monitoring and Aligner Treatment

At-Home Aligner Therapy Teleorthodontics paves the way for at-home aligner therapy. This section delves into how patients receive aligners through mail or digital channels and follow treatment protocols from the comfort of their homes. Regular check-ins and virtual appointments enable orthodontists to monitor progress and provide guidance remotely.

Advanced Monitoring Technologies The chapter explores how advanced monitoring technologies are incorporated into remote aligner treatments. From smartphone apps and wearable devices to AI-driven algorithms, these technologies enable patients to capture and share images of their progress. Orthodontists can then remotely assess the effectiveness of treatment and make necessary adjustments.

Challenges and Ethical Considerations

Patient Compliance and Education While teleorthodontics offers convenience, patient compliance and education remain pivotal. This section discusses strategies for ensuring that patients adhere to treatment protocols, understand the importance of proper aligner wear, and promptly communicate any concerns to their orthodontists.

Ethical Guidelines and Informed Consent The chapter addresses ethical considerations in teleorthodontics. It explores topics such as informed consent for remote treatments, the role

of orthodontists in supervising treatment remotely, and the importance of maintaining patient safety and satisfaction in the digital realm.

Future Innovations

Integration of AI and Virtual Reality The chapter concludes by shedding light on the future innovations that could further enhance teleorthodontics. The integration of artificial intelligence and virtual reality technologies could offer new avenues for personalized treatment planning, patient engagement, and treatment outcomes.

Conclusion

Chapter 12 unveils the transformative potential of teleorthodontics and remote aligner treatments. From virtual consultations and treatment planning to advanced monitoring technologies, these approaches revolutionize orthodontic care. While challenges and ethical considerations persist, the promise of teleorthodontics continues to grow, forging a future where convenience, precision, and patient-centered orthodontic treatments intersect.

Chapter 13: Blockchain Applicationsin Dental Records

Enhancing Data Security and Accessibility in Oral Healthcare

The integration of blockchain technology into dental records management marks a significant advancement in data security, privacy, and accessibility. This chapter delves into the world of blockchain applications in oral healthcare, showcasing how this decentralized and immutable technology is reshaping the way dental records are stored, shared, and utilized.

In the age of digitization, data security and accessibility have become paramount concerns in oral healthcare. This chapter delves into the critical realm of data protection, shedding light on how advanced technologies and robust protocols are harnessed to ensure the confidentiality, integrity, and availability of patient information in an increasingly digital landscape.

The Digital Transformation of Oral Healthcare

Digitalization has brought about a host of benefits to oral healthcare, from streamlined workflows to enhanced patient engagement. However, it has also introduced new challenges related to data security and accessibility that must be diligently addressed.

Data Security

Encryption: Utilizing encryption methods ensures that sensitive patient data remains unreadable by unauthorized parties.

Secure Storage: Adopting secure servers and cloud platforms protects patient data from breaches and cyberattacks.

Access Controls: Implementing stringent access controls limits data access to authorized personnel only.

Data Access Auditing: Regular auditing of data access logs helps identify and mitigate potential security breaches.

Cybersecurity Training: Educating dental professionals on cybersecurity best practices is essential to prevent breaches.

Data Accessibility

Interoperability: Ensuring that different systems can exchange and interpret data fosters seamless information sharing.

Patient Portals: Providing patients with secure online portals allows them to access their health records and treatment information.

Telehealth Platforms: Secure telehealth platforms facilitate remote consultations while maintaining data integrity.

Benefits of Data Security and Accessibility

Patient Trust: Ensuring data security fosters patient trust, encouraging open communication and informed decision-making.

Efficient Collaboration: Secure data accessibility enables efficient communication between dental professionals, specialists, and laboratories.

Research and Innovation: Access to secure patient data supports research and innovation in oral healthcare.

Challenges and Considerations

Constant Vigilance: The evolving nature of cyber threats requires ongoing monitoring and proactive security measures.

Regulatory Compliance: Compliance with healthcare regulations and privacy laws is vital to data protection.

Human Factor: Training dental professionals to recognize and respond to potential security threats is crucial.

Ethical and Legal Implications

Informed Consent: Patients should be informed about data collection, storage, and usage practices.

Data Ownership: Clarifying patient and provider rights and responsibilities regarding data ownership is essential.

Human-Technology Synergy

Professional Judgment: Technology enhances data security but does not replace the vigilance of dental professionals.

Continuous Learning: Staying updated on cybersecurity best practices is essential to safeguard data.

Forging a Secure Digital Frontier

In the digital era, data security and accessibility are pivotal for maintaining patient trust and ensuring efficient oral healthcare delivery. By employing robust cybersecurity measures, adopting secure platforms, and fostering a culture of data protection, the dental community can navigate the digital landscape while upholding ethical standards and patient confidentiality. As technology advances, the future of oral healthcare holds exciting prospects, promising a landscape where data security and accessibility coexist harmoniously to ensure safe, effective, and patient-centered care.

Understanding Blockchain Applications in Dental Records

Blockchain technology, renowned for its security features, has found a unique application in dental records management. This section introduces the concept of blockchain and explains how its decentralized and tamper-proof nature addresses challenges in data security, interoperability, and patient privacy.

Decentralized Patient Data Management

One of the primary applications of blockchain in dentistry lies in decentralized patient data management. This section explores how patient records, including treatment histories, radiographs, and appointments, can be securely stored on the blockchain. This decentralized approach ensures that patients' sensitive information remains private and can be accessed only by authorized parties.

Interoperability and Data Sharing

Blockchain technology addresses the issue of interoperability by providing a secure and standardized platform for data sharing across dental practices and healthcare providers. This section discusses how blockchain's distributed ledger ensures that patient records are consistent, accurate, and accessible to authorized stakeholders, fostering better collaboration and continuity of care.

Enhancing Data Privacy and Consent

Blockchain empowers patients with greater control over their data. Through smart contracts and cryptographic mechanisms, patients can provide explicit consent for data sharing and control who has access to their information. This section delves into how blockchain enhances data privacy and aligns with regulations such as the General Data Protection Regulation (GDPR).

Data Integrity and Verification

Blockchain's immutability ensures the integrity and authenticity of dental records. This section explores how the technology creates a permanent record of data changes, preventing unauthorized alterations and providing an audit trail. Dental professionals can confidently rely on accurate and trustworthy records for diagnosis, treatment planning, and research.

Challenges and Future Directions

While block-chain offers transformative benefits, challenges such as scalability, adoption barriers, and regulatory compliance exist. This section discusses potential solutions to these challenges and envisions a future where block-chain's potential is fully realized in dental records management.

Conclusion

Chapter 13 underscores the revolutionary potential of blockchain applications in dental records management. By enhancing data security, interoperability, and patient privacy, blockchain technology paves the way for a more efficient, transparent, and patient-centric approach to oral healthcare. While challenges remain, the promise of blockchain continues to shape the future of dental records, offering a glimpse into a more secure and interconnected healthcare landscape.

Chapter 14: Digital Implantologyand Guided Surgery

Precision and Innovation in Implant Dentistry

The integration of digital technology into implantology has revolutionized the way dental implants are planned, placed, and restored. This chapter delves into the realm of digital implantology and guided surgery, showcasing how these innovations enhance precision, predictability, and patient outcomes in the field of implant dentistry.

Implant dentistry has evolved into a realm of precision and innovation, transforming the landscape of tooth replacement with cutting-edge technologies that promise optimal outcomes and patient satisfaction. This chapter delves into the world of implant dentistry, shedding light on how advancements in technology have elevated precision, improved treatment planning, and reshaped the art of crafting perfect smiles through dental implantation.

The Era of Precision Implantology

Precision implant dentistry entails the use of advanced technologies to ensure the accurate placement, restoration, and long-term success of dental implants.

Digital Imaging

Cone Beam Computed Tomography (CBCT): CBCT provides three-dimensional images of the oral structures, aiding in precise treatment planning.

Virtual Implant Placement: Digital imaging allows for virtual implant placement, predicting optimal implant locations.

Guided Surgery: Computer-guided systems facilitate precise implant placement based on virtual planning.

Enhanced Treatment Planning

Digital Impressions: Intraoral scanners replace traditional molds, enabling accurate digital models for treatment planning.

Digital Wax-ups: Digital simulations help plan implant placements in harmony with the overall dental esthetics.

Virtual Mock-ups: Patients can visualize the final result before the actual implant procedure.

Implant Design and Materials

Custom Implants: 3D printing and CAD/CAM technology allow for custom implant designs tailored to patient anatomy.

Bioactive Materials: Implants coated with bioactive materials enhance osseointegration and long-term stability.

Digital Prosthesis Fabrication

CAD/CAM Restorations: Computer-aided design and manufacturing create precise prosthetic components.

Digital Workflow: Seamless communication between implantologists and dental technicians ensures accurate prosthetic fabrication.

Benefits of Precision and Innovation

Predictable Outcomes: Advanced planning and guided procedures result in more predictable implant outcomes.

Minimized Discomfort: Precise implant placement reduces post-operative discomfort and healing time.

Aesthetics: Digital simulations ensure implant placements harmonize with natural dental esthetics.

Challenges and Considerations

Technology Integration: Adopting new technologies requires training and initial investment.

Data Accuracy: Precise implant placement relies on accurate patient data from digital imaging.

Ethical and Patient-Centric Approach

Informed Consent: Patients must understand the benefits, risks, and alternatives of implant procedures.

Treatment Alternatives: Ethical decision-making involves presenting non-implant treatment options to patients.

Human-Technology Synergy

Clinical Expertise: Technology enhances precision but does not replace the judgment of dental professionals.

Continual Learning: Staying updated with implant dentistry advancements is essential for optimal patient care.

A New Horizon of Smiles

Precision and innovation have ushered in a transformative era in implant dentistry, allowing dental professionals to create smiles that are not only functional but also aesthetically pleasing. By harnessing advanced technologies to enhance treatment planning, implant design, and prosthetic fabrication, implantologists are sculpting a new horizon of dental care that prioritizes precision, patient outcomes, and the artistry of crafting perfect smiles. As the field continues to evolve, the future of implant dentistry holds exciting possibilities, promising a landscape where cutting-edge techniques and human expertise converge to create enduring smiles that radiate confidence and happiness.

Understanding Digital Implantology and Guided Surgery

Digital implantology leverages advanced technologies such as cone beam computed tomography (CBCT), intraoral scanning, and computer-aided design (CAD) to transform

implant procedures. This section introduces the concept of guided surgery, where digital planning and surgical guides are employed to ensure accurate implant placement and optimal prosthetic outcomes.

Virtual Treatment Planning and 3D Imaging

Digital implantology begins with virtual treatment planning and 3D imaging. This section explores how CBCT scans and intraoral scans are merged to create comprehensive 3D models of a patient's oral anatomy. Dental professionals can then virtually plan the implant placement, considering bone quality, available space, and prosthetic design.

Surgical Guide Fabrication and Precision

One of the cornerstones of digital implantology is the fabrication of surgical guides. These guides, created through CAD/CAM processes, provide a roadmap for implant placement with unparalleled accuracy. This section discusses how surgical guides enhance precision, minimize invasiveness, and contribute to shorter surgery times.

Implant Placement with Predictability

Guided surgery ensures a high level of implant placement predictability. By following the pre-planned trajectory and depth, dental professionals can achieve optimal implant

positioning. This section highlights how guided surgery minimizes complications, reduces the risk of postoperative discomfort, and contributes to successful osseointegration.

Restoration Planning and Prosthetic Outcomes

Digital implantology extends beyond surgery to prosthetic planning. This section explores how digital tools enable prosthetic design that complements the patient's anatomical features. Virtual planning ensures proper implant angulation, emergence profile, and occlusal function, leading to esthetic and functional prosthetic outcomes.

Challenges and Advancements

While digital implantology offers significant advantages, challenges such as learning curves, cost considerations, and the need for advanced training exist. This section discusses how continuous advancements in technology and education are addressing these challenges and expanding the accessibility of guided surgery.

Future Innovations and Conclusion

The future of digital implantology holds promising innovations. From artificial intelligence-assisted treatment planning to real-time guided surgery using augmented reality, this section envisions how technology will continue to refine implant procedures. Chapter 14 concludes

by highlighting the transformative impact of digital implantology on the field of implant dentistry.

In conclusion, Chapter 14 underscores the transformative potential of digital implantology and guided surgery. By merging digital planning, surgical guides, and advanced imaging, implant procedures become more precise, efficient, and patient-centered. While challenges persist, the promise of digital implantology continues to reshape the landscape of implant dentistry, offering a glimpse into the future of enhanced implant outcomes and patient satisfaction.

Chapter 15: Bioprinting and Tissue Engineering in Dentistry

Revolutionizing Restorative and Regenerative Dentistry

The convergence of bioprinting and tissue engineering has ushered in a new era of possibilities in restorative and regenerative dentistry. This chapter delves into the realm of bioprinting and tissue engineering applications in dentistry, highlighting how these cutting-edge technologies are reshaping the way dental tissues are repaired, regenerated, and restored.

Restorative and regenerative dentistry has entered a new era of innovation, where advancements in materials, techniques, and technologies are transforming the way dental professionals approach tooth repair, replacement, and tissue regeneration. This chapter delves into the dynamic world of restorative and regenerative dentistry, shedding light on how innovation is reshaping treatment modalities, enhancing patient outcomes, and unlocking the potential for natural and lasting oral health restoration.

The Evolution of Restoration and Regeneration

Restorative and regenerative dentistry encompasses a wide range of procedures aimed at repairing damaged tissues, replacing missing teeth, and promoting tissue regeneration.

Advanced Materials

Biocompatible Materials: Biomaterials closely mimic natural tooth structures, ensuring harmonious integration.

Smart Materials: Self-healing materials and bioactive agents promote tissue regeneration and longevity.

Digital Impressions: Intraoral scanners capture precise digital models for accurate restorations.

Digital Workflow: CAD/CAM technology enables efficient design and fabrication of restorations.

Implant Innovations

Osseointegration Enhancement: Surface modifications and coatings improve implant stability and osseointegration.

Custom Implants: 3D printing and CAD/CAM technology allow for patient-specific implant designs.

Tissue Regeneration

Growth Factors: Bioactive agents stimulate tissue growth and healing for periodontal and bone regeneration.

Stem Cell Therapies: Stem cells hold promise for regenerating dental pulp and periodontal tissues.

Benefits of Innovation

Enhanced Aesthetics: Advanced materials and techniques create restorations that seamlessly blend with natural teeth.

Longevity: Biomimetic materials and regenerative therapies promote long-term restoration and tissue health.

Minimized Discomfort: Minimally invasive techniques and innovative materials reduce patient discomfort.

Challenges and Considerations

Technological Mastery: Dental professionals must master new techniques and technologies for optimal outcomes.

Regulatory Compliance: Innovations must align with regulatory standards for patient safety.

Ethical and Patient-Centric Approach

Informed Consent: Patients should understand the benefits, risks, and alternatives of innovative treatments.

Evidence-Based Practice: Ethical decision-making involves adopting innovations with demonstrated efficacy.

Human-Technology Synergy

Clinical Judgment: Innovation complements clinical expertise but does not replace it.

Continuous Learning: Staying updated with restorative and regenerative advancements is essential for optimal patient care.

The Dawn of Dental Transformation

Restorative and regenerative dentistry's integration with innovation signifies a transformative journey towards natural, functional, and lasting oral health restoration. By harnessing the power of advanced materials, digital workflows, and regenerative therapies, dental professionals are rewriting the narrative of dental care, emphasizing precision, aesthetics, and tissue regeneration. As innovation continues to flourish, the future of restorative and regenerative dentistry holds exciting possibilities, promising a landscape where dental treatments are not just functional, but also artistic expressions of science and craftsmanship, ensuring smiles that radiate health, confidence, and vitality.

Understanding Bioprinting and Tissue Engineering in Dentistry

Bioprinting combines advanced 3D printing technologies with tissue engineering principles to create biocompatible constructs that mimic natural dental tissues. This section introduces the concept of bioprinting and discusses its potential in restoring damaged dental tissues and regenerating functional structures.

Biomaterials and Scaffold Design

The success of bioprinting lies in the selection of appropriate biomaterials and scaffold designs. This section explores how biocompatible materials, such as hydrogels and ceramics,

are used as building blocks for bioprinted constructs. Scaffold design is crucial for creating structures that support cell growth, nutrient diffusion, and tissue integration.

Bioprinting Techniques and Applications

Bioprinting encompasses various techniques, including extrusion-based, inkjet-based, and laser-assisted printing. This section delves into these techniques and their applications in dental tissue engineering. From bioprinting dental pulp for pulp regeneration to creating customized dental implants, the possibilities are extensive.

Dental Tissue Regeneration and Repair

Bioprinting and tissue engineering offer innovative solutions for dental tissue regeneration and repair. This section discusses how bioprinted constructs can replace damaged dental tissues, including enamel, dentin, and alveolar bone. Additionally, the integration of growth factors and stem cells enhances tissue regeneration potential.

Challenges and Ethical Considerations

While bioprinting holds immense promise, challenges such as achieving appropriate biomimicry, scalability, and regulatory approval need to be addressed. This section discusses these challenges and explores ethical considerations surrounding the use of bioprinted tissues, including patient consent and safety.

Future Directions and Conclusion

The future of bioprinting in dentistry is promising, with advancements in bioprinting technology, materials, and techniques. This section envisions how bioprinting could evolve, from creating personalized dental implants to regenerating entire teeth. Chapter 15 concludes by underscoring the transformative potential of bioprinting and tissue engineering in dentistry.

In conclusion, Chapter 15 underscores the groundbreaking impact of bioprinting and tissue engineering on the field of dentistry. By combining technological innovation with tissue regeneration principles, these approaches offer the potential to restore and regenerate dental tissues with remarkable precision. While challenges remain, the promise of bioprinting continues to shape the future of dental restorative and regenerative treatments, offering a glimpse into a new era of dental healthcare.

Chapter 16: Wearable Tech and Smart Devices in Oral Health

Empowering Oral Care Through Innovation

The integration of wearable technology and smart devices into oral health practices has ushered in a transformative era of personalized care and preventive approaches. This chapter delves into the world of wearable tech and smart devices in oral health, showcasing how these innovations empower individuals to monitor, manage, and optimize their oral well-being.

Innovation has emerged as a driving force in the realm of oral care, revolutionizing the way dental professionals diagnose, treat, and engage with patients. This chapter delves into the dynamic landscape of oral care innovation, shedding light on how cutting-edge technologies, novel approaches, and forward-thinking strategies are empowering dental practitioners, enhancing patient experiences, and shaping the trajectory of dental wellness for years to come.

The Power of Innovation in Oral Care

Innovation in oral care encompasses a broad spectrum of advancements that span diagnostic tools, treatment methods, patient communication, and practice management.

Technological Advancements

AI and Machine Learning: Artificial intelligence analyzes vast datasets to aid in diagnostics and treatment planning.

Telehealth: Virtual consultations and remote monitoring enhance patient access to dental expertise.

3D Printing: Customized dental prosthetics and orthodontic devices are fabricated with precision.

Enhanced Diagnostics

Digital Imaging: Cone beam computed tomography (CBCT) provides detailed 3D images for precise diagnosis.

Saliva Diagnostics: Non-invasive tests in salivary diagnostics aid in early disease detection.

Treatment Personalization

Precision Dentistry: Tailoring treatments to individual patient characteristics maximizes effectiveness.

Regenerative Therapies: Stem cells and growth factors promote tissue healing and regeneration.

Patient-Centered Care

Digital Communication: Online platforms enhance patient engagement, education, and appointment scheduling.

Educational Resources: Patients access digital materials for oral health information and preventive care.

Benefits of Innovation

Early Intervention: Advanced diagnostics and monitoring tools enable early detection of oral health issues.

Enhanced Outcomes: Personalized treatments and regenerative therapies lead to optimal results.

Efficiency: Digital workflows streamline processes, reducing treatment time and resource usage.

Challenges and Considerations

Adaptation: Embracing new technologies requires dental professionals to continually update their skill sets.

Ethical and Regulatory Compliance

Informed Consent: Patients should be informed about the use of innovative technologies in their care.

Data Privacy: Protecting patient data in the digital realm is a critical ethical responsibility.

Human-Technology Synergy

Clinical Judgment: Innovation complements clinical expertise but does not replace it.

Lifelong Learning: Staying informed about advancements is key to harnessing innovation effectively.

Forging a Brighter Path Forward

Innovation's integration into oral care marks a transformative shift towards patient-centered, efficient, and precise dental wellness. By embracing technological breakthroughs, customized treatments, and enhanced patient communication, dental professionals are carving a path towards a future where oral health is not just preserved but elevated. As innovation continues to evolve, the horizon of oral care holds exciting possibilities, promising a landscape where dentistry transcends traditional boundaries, and where the potential for healthier smiles is realized through the synergy of expertise and cutting-edge technology.

Understanding Wearable Tech and Smart Devices in Oral Health

Wearable technology and smart devices are reshaping the way individuals engage with their oral health. This section introduces the concept of wearable tech and smart devices, highlighting their potential to enhance oral care routines, provide real-time insights, and foster proactive oral health management.

Smart Toothbrushes and Oral Hygiene Monitoring

Smart toothbrushes have emerged as tools that provide real-time feedback on brushing habits. This section explores how sensors and connectivity features in smart toothbrushes track brushing duration, pressure, and coverage. By offering insights into oral hygiene practices, these devices encourage improved brushing techniques and promote better gum health.

Oral Health Trackers and Data Insights

Wearable oral health trackers, often worn as devices or integrated into accessories, monitor various oral health parameters. This section discusses how these trackers can measure factors such as oral pH, temperature, and salivary composition. By collecting data over time, users and healthcare professionals gain valuable insights into potential issues and trends.

Smart Flossers and Interdental Cleaning

Smart flossers and interdental cleaners incorporate technology to enhance interdental cleaning practices. This section explores how these devices guide users in effectively cleaning between teeth, promoting gum health and preventing cavities. The integration of technology into interdental care empowers individuals to maintain optimal oral hygiene.

Oral Health Coaching and Remote Monitoring

Smart devices in oral health offer coaching and monitoring features that extend beyond individual use. This section discusses how wearable tech can connect to mobile apps or cloud platforms, allowing dental professionals to remotely monitor patients' oral health progress, offer personalized recommendations, and track treatment adherence.

Challenges and Ethical Considerations

While wearable tech and smart devices offer significant benefits, challenges such as data security, accuracy, and patient privacy exist. This section explores how to address these challenges and maintain ethical standards in collecting, sharing, and using oral health data.

Future Innovations and Conclusion

The future of wearable tech and smart devices in oral health holds exciting possibilities. This section envisions advancements such as real-time disease detection, AI-powered oral health analysis, and integration with electronic health records. Chapter 16 concludes by underscoring the transformative impact of wearable tech and smart devices in oral health management.

In conclusion, Chapter 16 underscores the transformative potential of wearable technology and smart devices in oral health. By providing real-time insights, promoting preventive practices, and enabling remote monitoring, these innovations empower individuals to take proactive control of their oral well-being. While challenges persist, the promise of wearable tech continues to shape the future of oral health, offering a glimpse into a more connected and personalized approach to dental care.

Chapter 17: Teleprosthodontics and Removable Appliances

Revolutionizing Prosthodontic Care Through Telehealth

The integration of telehealth into prosthodontics has paved the way for teleprosthodontics, a transformative approach that redefines how removable dental appliances are designed, delivered, and managed. This chapter delves into the realm of teleprosthodontics and its applications in removable appliances, showcasing how virtual consultations, digital design, and remote monitoring are reshaping the landscape of prosthodontic care.

Understanding Teleprosthodontics and Removable Appliances

Teleprosthodontics represents a paradigm shift in prosthodontic care. This section introduces the concept of teleprosthodontics and explores how digital technologies are harnessed to provide virtual consultations, design removable appliances, and monitor treatment progress remotely.

Virtual Consultations and Treatment Planning

Teleprosthodontics begins with virtual consultations that transcend geographical barriers. This section discusses how patients can connect with prosthodontists through video calls, share oral images, and discuss their prosthetic needs. Virtual consultations empower patients to receive expert advice and explore treatment options without the need for in-person visits.

Digital Design and Fabrication

One of the cornerstones of teleprosthodontics lies in digital design and fabrication of removable appliances. This section explores how intraoral scans and digital impressions replace traditional molds. Prosthodontists can use digital software to design dentures, partials, and other removable appliances with precision, ensuring optimal fit and aesthetics.

Remote Monitoring and Adjustment

Teleprosthodontics extends beyond initial design to ongoing treatment monitoring. This section discusses how patients can receive remote guidance on denture adjustments, relines, and other prosthetic modifications. Virtual follow-ups allow prosthodontists to assess fit, comfort, and functionality without requiring patients to travel.

Challenges and Ethical Considerations

While teleprosthodontics offers significant benefits, challenges such as accurate remote assessment, patient comfort, and ethical use of technology need to be addressed. This section discusses strategies for overcoming these challenges and ensuring that teleprosthodontic practices align with ethical standards.

Future Directions and Conclusion

The future of teleprosthodontics holds promising innovations. From AI-driven treatment planning to real-time adjustments through augmented reality, this section envisions how technology will continue to refine prosthetic procedures. Chapter 17 concludes by highlighting the transformative impact of teleprosthodontics on the field of removable appliances.

In conclusion, Chapter 17 underscores the groundbreaking impact of teleprosthodontics and its applications in removable appliances. By combining virtual consultations, digital design, and remote monitoring, prosthodontic care becomes more accessible, personalized, and patient-centric. While challenges remain, the promise of teleprosthodontics continues to reshape the landscape of removable appliance treatments, offering a glimpse into the future of convenient and effective prosthodontic care.

Chapter 18: Ethics and Regulationsin Digital Dentistry

Navigating the Ethical Landscape of Technological Advancements

As digital dentistry continues to revolutionize oral healthcare, it brings forth a multitude of ethical and regulatory considerations that demand careful attention. This chapter delves into the intricate interplay between ethics, regulations, and digital dentistry, exploring how practitioners navigate the ethical challenges posed by emerging technologies and ensure patient safety and well-being.

Understanding the Ethical Framework of Digital Dentistry

The introduction of digital technologies in dentistry introduces novel ethical complexities. This section delves into the fundamental ethical principles of autonomy, beneficence, non-maleficence, and justice, and how they apply to the integration of digital tools in patient care and practice management.

Informed Consent and Patient Autonomy

In the context of digital dentistry, informed consent takes on new dimensions. This section discusses the importance of transparent communication with patients regarding the use of digital technologies, data collection, and potential risks. Upholding patient autonomy requires practitioners to ensure that patients fully understand and consent to digital procedures and their implications.

Data Privacy and Security

The digital realm introduces concerns about patient data privacy and security. This section explores the ethical responsibility of dental professionals to safeguard patient information, adhere to data protection regulations, and adopt secure digital practices to prevent unauthorized access or breaches.

Emerging Technologies and Transparency

As new technologies emerge, practitioners must ensure transparency in their implementation. This section discusses the ethical considerations of introducing cutting-edge technologies, such as AI and bioprinting, and maintaining clear communication with patients about potential benefits, risks, and limitations.

Balancing Innovation and Evidence-Based Practice

The pursuit of innovation must be tempered by evidence-based practice to ensure patient safety and efficacy. This section highlights the ethical duty of practitioners to critically assess the scientific validity of new digital tools before integrating them into patient care.

Regulatory Landscape and Compliance

Adhering to Professional Standards

Dental practitioners have a professional obligation to abide by ethical guidelines and industry standards. This section discusses the importance of following guidelines set by dental associations and regulatory bodies to ensure ethical digital practices.

Regulatory Compliance

The integration of digital technologies often intersects with regulations related to medical devices and data protection. This section explores the legal and ethical considerations associated with using FDA-approved devices, adhering to HIPAA regulations, and maintaining patient confidentiality.

Conclusion: Ethical Excellence in a Digital Era

Chapter 18 underscores the crucial role of ethical considerations and regulatory compliance in the era of digital dentistry. As technological advancements continue to shape oral

healthcare, dental professionals must navigate the ethical landscape with mindfulness, transparency, and a commitment to patient well-being. By upholding ethical standards, practitioners can ensure that the digital transformation of dentistry remains a force for positive change, offering advanced care while maintaining the utmost respect for patients' rights and safety.

Chapter 19: Future Challenges and Opportunities

Navigating the Evolving Landscape of Digital Dentistry

The dynamic world of digital dentistry presents a horizon filled with both challenges and opportunities that will shape the future of oral healthcare. This chapter delves into the multifaceted aspects of the digital dentistry landscape, exploring the potential challenges that lie ahead, while also highlighting the exciting opportunities for innovation, improved patient care, and professional growth.

Anticipating Technological Advancements

The rapid pace of technological advancement is both an opportunity and a challenge. This section delves into the potential for new technologies, such as AI, nanotechnology, and virtual reality, to revolutionize dental practice. While exciting, these advancements may also necessitate ongoing education and adaptation to remain at the forefront of the field.

Interdisciplinary Collaboration

Digital dentistry's expansion encourages interdisciplinary collaboration. This section explores how dentists, engineers, data scientists, and other professionals must collaborate to harness the full potential of digital tools. This cross-disciplinary approach offers opportunities for creative problem-solving and the development of innovative solutions.

Ensuring Equity in Access

While digital dentistry offers numerous benefits, ensuring equitable access to these advancements is a challenge. This section discusses the importance of addressing socioeconomic disparities to prevent a digital divide in oral healthcare, and how digital technologies can be harnessed to bridge these gaps.

Ethical Dilemmas and Regulation

As the digital landscape evolves, ethical dilemmas and regulatory complexities will emerge. This section explores the potential ethical challenges posed by AI diagnosis, data privacy concerns, and bioprinting ethics. Dental professionals must work alongside regulators to develop guidelines that maintain patient safety and ethical standards.

Patient-Centered Care and Education

Digital dentistry's future lies in personalized patient care. This section highlights how advancements in patient education, treatment planning, and engagement will shape the patient-provider relationship, empowering patients to actively participate in their oral health management.

Enhancing Practice Management

Digital technologies can streamline practice management and enhance patient experiences. This section explores how tools like electronic health records, appointment scheduling apps, and virtual communication platforms will transform the way dental practices operate, leading to improved efficiency and patient satisfaction.

Conclusion: Embracing the Future with Resilience

Chapter 19 underscores the dual nature of the digital dentistry landscape – a realm filled with challenges and opportunities. By embracing innovation, interdisciplinary collaboration, and ethical considerations, dental professionals can navigate the evolving landscape with resilience and determination. The future of digital dentistry offers the potential to elevate patient care, expand professional horizons, and create a truly patient-centered oral healthcare experience.

Chapter 20: The Road Ahead: Shaping the Future of Digital Dentistry

Charting a Course for Transformative Evolution

As the journey through the realm of digital dentistry continues, it's essential to envision the path forward and the profound impact it will have on the field of oral healthcare. This final chapter delves into the exciting possibilities that lie ahead, offering insights into how practitioners, researchers, and stakeholders can collectively shape the future of digital dentistry for the betterment of patients and the profession.

Integration of Emerging Technologies

The future of digital dentistry rests upon the integration of emerging technologies. This section explores how AI, virtual reality, bioprinting, and other innovations will intertwine to create a comprehensive and patient-centric oral healthcare experience.

Data-Driven Precision Care

Data-driven insights will revolutionize clinical decision-making. This section discusses how the analysis of large datasets and predictive modeling will enable practitioners to offer personalized treatment plans and interventions based on individual patient profiles.

Enhanced Patient Engagement

Digital tools will empower patients to become active participants in their oral health management. This section explores how wearable tech, mobile apps, and interactive platforms will enhance patient engagement and adherence to treatment plans.

Continued Education and Training

With the rapid pace of technological advancement, ongoing education and training are crucial. This section emphasizes the need for dental professionals to embrace lifelong learning to stay current and proficient in the ever-evolving digital landscape.

Ethical Innovation and Regulation

As digital dentistry evolves, ethical considerations will remain at the forefront. This section emphasizes the importance of ethical innovation and proactive regulatory measures to ensure that patient safety, privacy, and well-being are prioritized.

Global Accessibility and Equity

Efforts to make digital dentistry accessible on a global scale will be vital. This section discusses how digital technologies can bridge geographical gaps, allowing underserved communities to benefit from advanced oral healthcare solutions.

Collaborative Research and Development

The future of digital dentistry requires collaboration among researchers, practitioners, technologists, and patients. This section highlights the significance of collective efforts in pushing the boundaries of what's possible and addressing the challenges that arise.

Conclusion: Pioneering the Future of Digital Dentistry

Chapter 20 serves as a call to action, urging dental professionals to actively shape the trajectory of digital dentistry. By embracing innovation, ethical practices, and collaborative partnerships, practitioners can lead the transformation of oral healthcare into a realm that prioritizes precision, accessibility, and patient-centered care. The journey ahead is an exciting one, where the fusion of digital technology and oral healthcare will redefine possibilities and elevate the standard of dental practice for generations to come.

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Chapter - 1
L.E.D. (Light Emitting Diode) Appliance for Breaking Thumb Sucking Habit (Paediatric Dentistry)

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Chapter - 1

L.E.D. (Light Emitting Diode) Appliance for Breaking Thumb Sucking Habit (Paediatric Dentistry)

Dr. K. Kanimozhi

Abstract

Habits are routine behaviors that occur spontaneously and unconsciously, playing a crucial role as either positive, like regular exercise, eating healthy etc., or negative, like smoking, consumption of alcohol etc., Oral habits, such as thumb sucking, lip biting, and teeth grinding etc.,specifically affect the oral cavity and cause harm to the dental structures. These habits carry psychological implications, necessitating a holistic treatment approach that addresses both physical and psychological aspects, particularly in children.

The habits confined to the oral cavity are termed as Oral Habits and among the oral habits, the most noticed oneis thumb sucking, and it is seen mostly in neonates and declines by the first year of life. However, few children continue to suck their thumb for the pleasure derived by sucking, or to overcome their psychological discomfort even beyond the age of four, which requires intervention. Treating this habit requires a multifaceted approach that includes positive reinforcement, behavioral changes, and parental assistance.

In the present digital era, technology is constantly upgrading and redefining every aspect of our life at an unimaginable rate. The field of dentistry is also growing rapidly and is marking a remarkable stride in the present decade. Awareness of the need for proper oral hygiene and patients demand for aesthetically appealing smiles is making a notable impact among dentists in inventing better treatment approaches, dental materials, equipment, etc. These technological advancements are playing a significant role in delivering better treatment outcomes and patient satisfaction.

Many conventional approaches have been in practice for many decades concerning thumb-sucking habits. These approaches commonly use physical barriers for treatment, which causes trauma to the oral structures and leads to infections. However, with technology, many new innovative treatment approaches are being used, which act as friendly reminders to the child rather

than as punishments. One such is the habit-breaking appliance using L.E.D., which has multiple benefits for the treating dentist and the patient. This article explores this recent advancement in the field of paediatric dentistry.

Keywords: Paediatric dentistry, advancements, thumb sucking, habits, L.E.D. appliance

"Your habits will determine your future" -Jack Canfield

Introduction

Since our infancy, we humans tend to behave based on the actions we are most used to and familiar with. Such actions reflect our thoughts and serve to express our emotions. These actions/behaviors which become a part of our life are termed "Habits". American Psychological Association defines a *habit* as "a well-learned behavior or automatic sequence of behaviors that are relatively situation specific, that, over time, it becomes motorically reflexive and independent of motivational or cognitive influence, that it is performed with little or no conscious intent" [1]. Habits could be useful or harmful (William James, 1923) and, those habits which occur during infancy, mostly starts, and ends spontaneously [2].

Human beings, even before we start to communicate verbally, we express our emotions through our facial expressions and body language. All our emotions, like love, surprise, anger, happiness, frustration, anxiety, etc., are expressed through our eyes and mouth. Our mouth (oral cavity) exhibits these emotions by biting the tongue and nails, clenching the teeth, etc. These habits, which are confined to the oral cavity, are termed "Oral habits", and they have been classified into two major groups, namely, 1) Acquired and 2) Compulsive. Acquired habits refer to the habits which are easily gotten rid of, once when the child grows up (Finn, 1998). In contrast, compulsive oral habits include behaviors that are so engraved in the child that it creates a sense of irritation and anger when being asked to withdraw from the habit (Finn, 1998).

The various oral habits are,

1) Thumb/digit/finger sucking
2) Tongue thrusting
3) Lip licking and biting
4) Mouth breathing
5) Bruxism
6) Nail biting (onychophagia)
7) Cheek biting

8) Self-injurious habits like frenum thrusting, picking the gingiva, pencil biting, bobby pin opening, and insertion of sharp objects on the gingiva.

Though these habits provide psychological relief to the individual, they lead to deleterious effects on the dentoalveolar structures, requiring interventions. Despite being well aware of the benefits of breaking the habit, the steps to quit the habit remains a huge challenge among many individuals who are in the process of quitting the habit. It takes a lot of hard work and determination to give up them.

Thumb sucking habit

The most commonly seen as well the first observed habit among preschool children is thumb sucking. This habit is categorized as non-nutritive sucking and begins during the intrauterine period of twenty-nine weeks of age. During infancy, this habit mostly happens while sleeping and declines by the end of the first year [3]. Thumb sucking habit is normal and reversible within four years of age. However, if prolonged, it will damage dentoalveolar structures, which would require interventions.

Fig 1: A child with thumb sucking habit
Source: sheibaninia.net

Definition

Gellin (1970) defines thumb sucking as the "placement of thumb in varying depths into the mouth" [4].

Phases of thumb sucking habit (According to Moyer)

Phase 1-includes children who have the habit of sucking the thumb right from birth till three years of age. This stage is to be absolutely normal and sub-clinically significant. Hence, any preventive measure like placing an oral pacifier is suggested to stop this habit.

Phase 2-is clinically significant in children between three to seven years of age. It is highly recommended to observe changes in the child's dentoalveolar structures and begin the treatment accordingly.

Phase 3-refers to the child in whom the habit persists beyond the age of seven and is more serious, as it requires both dental and psychotherapy to treat the child. This phase is also called "intractable sucking", as it is hard to control the habit [9].

Epidemiology

In general, oral habits among the mixed dentition population have been identified to be more common in children from south India (30%), unlike the children in North India (3%) [5]. Concerning thumb-sucking habit, children all over the world irrespective of their socioeconomic status, have been prone to it [6], with a prevalence between 13% and 100% in some countries [7]. Traisman AS and Traisman HS have reported in their study that the average age of stopping the thumb-sucking habit is 3.8 years. However, other studies have reported that up to 20% of children have continued until four years of age [8].

Aetiology

Psycho-social factors associated with the loss of emotional security and comfort of the child, such as gender, age, birth order, social status, maternal age, maternal education, and maternal occupation, are the factors leading to thumb-sucking [9].

Pathophysiology

The adverse effects caused due to thumb sucking habit depend on the factors such as frequency, intensity and duration of the habit, which ultimately leads to deformations in the dentoalveolar and skeletal structures. [8]. These deformations are manifested in the form of such as proclained maxilla, increased arch length, increased S.N.A. angle, retroclined mandible, posterior crossbite, anterior open bite, narrow nasal vault, high palatal arch, lip incompetence, hypotonic upper lip and hyperactive lower lip, lower tongue position, increased trauma to maxillary incisors, atypical root resorption in the primary central incisor [10]. Apart from these, defects in speech and damage to the thumb used in sucking are also seen in patients with thumb-sucking habit [9].

Diagnosis

Children with the habit of thumb sucking are typically identified by observing their thumb, which is seen as a reddened, explicitly clean but chapped and short nail (clean dishpan thumb), and by enquiring about their history of the habit through questions such as the frequency, duration, and the intensity of thumb sucking. It is also essential to rule out the child's emotional status [9] and look for other dentoalveolar changes.

Management

"It is easier to prevent habits than to break them"-Benjamin Franklin

The first line of management is to counsel the child to quit the habit and make them understand the ill effects of thumb sucking habit. However, in those cases where treatment/intervention is indicated, the child must be explained properly about the treatment to get complete cooperation from the child throughout the treatment phase [9].

The various modes of management are:

1) **Counselling:** This is the widely used approach rather than a method, where the dentist explains to the child the changes which are occurring due to thumb sucking by showing them clinical photographs of those who have had the habit and by showing the child stone models with the damages in the dental structures. In addition, the child is also explained the impact on the physical appearance due to this habit and the possibility of lack of acceptance in social settings. The above-said approach works best in patients who are quite grown up, sensitive and cooperative [9].
2) **Psychological approach:** In this approach, the child is made to sit in front of a large mirror while sucking the thumb and asked to observe themselves in the mirror [9]. This method helps the child become more conscious of how it would be for the opponent to see them while sucking the thumb.
3) **Positive reinforcement (Reward system):** This method is executed by appreciating the child when the child refrains from the habit during the selected period. On doing so, the child is rewarded with stars as appreciation, or they are appreciated verbally [8]. Also, a personalized calendar is placed in a prominent location in the house to keep track of the habit-free time of the child, which is rewarded accordingly [11]. However, this method involves the active participation of the parent or the caretaker to keep track of the child.
4) **Reminder therapy:** This method involves placing waterproof tape or an adhesive bandage on the thumb, which will constantly keep reminding the child to refrain from the habit whenever they attempt to suck the thumb. Besides, shirts that cover the hand or plastic sleeves covering the thumb, which is available, are also used. Commercially available bitter-tasting substances applied over the thumb, like nail polish, can also be used. However, this method has been considered more as a punishment than a healthy way to

eliminate the habit. When this therapy is used along with the reward system, it has been identified to fetch better results [11].

5) **Adjunctive therapy or appliance therapy:** This therapy is introduced only after the child cannot refrain from this habit of sucking the thumb. This method creates a physical interruption with extraoral aids like an elastic band-aid or other equivalent material, which restrains the thumb from reaching the mouth. These bands are ideally placed during the night, and the parents are well informed that this method is followed only to prevent the child from this habit permanently and not as a punishment [11]. Orthodontic interventional appliances such as palatal cribs, spurs, palatal bars, hay rakes, and cagetype appliances are also used [8].

Advancements in treating thumb-sucking patients using electronic devices

Though there are many traditional methods and appliances available to break the thumb-sucking habit, the usage of these appliances is also known to cause difficulties during mastication, tongue ulcerations, and altered phonetics, which makes the treatment more like a punishment rather than acting as a healthy reminder to the child [12]. Additionally, using these appliances affects the child aesthetically too, causing stress.

But, in the present digital era, where technology is growing by leaps and bounds, dentistry has not been an exception. Moreover, considering the drawbacks of using traditional appliances, several habit-breaking appliances are being invented, including electronic devices that remind patients to refrain from the habits.

History of using electronic devices in habit-breaking appliances

Modified RURS elbow guard with revised three-alarm system

This system was introduced by Raghavendra M Shetty *et al*. in the year 2015 as a modified version of the existing "three-way alarm system", which uses the RURS elbow guard. Norton and Gellin introduced the original three-alarm system. Considering the constraints in using the original elbow guard, this revised RURS elbow guard was used to treat a 9-year-old child with a thumb-sucking habit successfully. On extra-oral examination, this child had presented with mild open bite intra-orally and a clean chapped left thumb with keratinization and callus formation on extra-oral examination.

In this modified elbow guard, a musical chip attached to a speaker was attached on the outer side of the elbow guard, and the inner side had its switch

button. The elbow guard was covered with a zip and attached with a Velcro strap. So, whenever the child attempts to suck the thumb, the switch button would get activated as it is getting pressed by the pressure caused when the child is taking the elbow closer to the mouth for sucking; as a result of this, music plays, which acts as a reminder for the child to refrain from sucking. The device was well adapted by the child as it ensured a positive way to avoid the habit. Also, being an extra-oral device, it helped avoid many complications arising from an orthodontic appliance [13].

Fig 2: Child wearing the modified elbow guard [13]
Source: Original article written by Raghavendra M Shetty *et al*.

Electronic wristwatch with alarm

This appliance was designed and patented by Krishnappa et al. in 2014 and published in 2016. This invention has successfully treated an 8-year-old child with a prolonged thumb sucking habit. On intra-oral examination, the child had presented with a decrease in overbite and open bite with mildly proclined maxillary incisors. This appliance used the simple concept of using a custom-made attractive wristwatch with an alarm attached to an extraoral appliance. Furthermore, this appliance was made to wear on the finger, which the child used for sucking. So, whenever the child takes their thumb to suck, the alarm starts working as a reminder for the child to refrain from the habit. The main components of this appliance were 1) the extraoral part, which has the four contact heads and is fixed to the acrylic ring (as seen in Figure 3), and 2) the reminder part, which is the wristwatch with the alarm and its battery which is connected to the appliance by wires. This appliance effectively helped the child cease the habit within five months; however, to prevent relapse, the child was made to continue wearing the appliance for another six

months. During this entire treatment phase, the child had felt very comfortable using the appliance and did not encounter any teasing by peers as well. This habit breaking appliance was also cost-effective compared to the other appliances, thereby making it more feasible for the parent to have a positive attitude to get their child treated without causing any financial burden to them. [14].

Fig 3: Electronic wrist watch with alarm [14]
Source: Original article written by Krishnappa *et al*.

Habit-breaking appliance using light emitting diode (L.E.D.)

In the present era, where technology has reached every nook and corner, People have developed the tendency to seek treatment options that use recent technology apart from saving time and being attractive and affordable. Even in dentistry, we observe an increasing trend in patients seeking treatment for better aesthetics. Matching the present trend, many new appliances have been invented for breaking oral habits and one such being the habit-breaking appliance using Light Emitting Diode (L.E.D.).

Introduction

This habit-breaking appliance using L.E.D. was introduced by Sahu A and Shyagali TR in 2017. The appliance used a novel approach in treating the child with thumb-sucking habit using a Hawley's appliance with a short labial bow and Adam's clasp on the first maxillary molars. An electric circuit with L.E.D. and a switch were attached to this appliance in such a manner that the light would glow whenever the child attempted to suck the thumb. Thus, creating a reminder to the child to refrain from the habit.

Patient description

A 12-year-old female child who had presented with the chief complaint of protruded maxillary incisors, which had occurred due to a prolonged thumb-sucking habit, had been treated using this appliance [12].

Designing the habit-breaking appliance

1) An accurate impression of the maxillary arch was made using alginate, and its models were prepared.
2) A regular Hawley's retainer was fabricated with a short labial bow and Adam's clasp on the first molars.
3) The acrylic on the palatal portion of Hawley's retainer was thinned out to the maximum.
4) Using an electrician's help, an electric circuit was formed using a coat-size battery, a small Light Emitting Diode (L.E.D.) colour bulb, and a small piece of plastic-coated copper wire with an on/off switch or feather touch switch.
5) Then, this circuit was placed on Hawley's appliance and acrylized using cold-cured resin. Except for the L.E.D. colour bulb and the on/off switch, all other circuit parts were embedded within the acrylic portion of the appliance (figure 4).
6) During acrylization, the L.E.D. bulb was placed just behind the upper central incisors, and the on/off switch was placed at the point where the thumb would most likely come in contact while the child is attempting to suck. However, extreme caution was taken during acrylization to prevent the exposure of the battery and wire in the oral environment.
7) Following this, the appliance was finished and polished.
8) This appliance was inserted into the patient's mouth and checked for proper fitting (figure 5)

Fig 4: Model of the appliance using L.E.D [12]
Source: Original article written by Sahu and Shyagali

Fig 5: Patient using the L.E.D. habit-breaking appliance [12]
Source: Original article written by Sahu and Shyagali

Duration of using the appliance

Similar to that of any other conventional habit-breaking appliance.

Discussion

Many infants and children have the habit of sucking the thumb since infancy, and few start even when they are in the womb. Thumb sucking occurs due to hunger, sleepiness, tiredness, boredom, stress, etc.; by doing so, they enjoy the psychological comfort it provides.

Any child with oral habits is always encouraged to break the habit through positive psychological approaches, which would be as reminders rather than punishments. So, even the use of appliances is indicated only as a last resort. This L.E.D. habit-breaking appliance works by providing gentle and friendly reminders to the child and acts more psychologically in helping the child to get rid of this habit completely. Unlike conventional appliances, which work more mechanically by serving as a barrier for the child to suck the thumb. This L.E.D. Appliance benefits in two ways, 1) since the child is wearing this appliance intraorally, it would always be a constant reminder that they are in the process of quitting the habit 2) as the L.E.D. begins to glow every time the child attempts to suck its thumb, this glowing light in the mouth being visible to all, would make the child feel more conscious and would provoke the child stop the sucking immediately.

Pros of this appliance

1) Easy to fabricate.
2) Hassle-free maintenance.
3) Cost-effective.
4) Does not cause any harm to the oral structures.
5) Acts more psychologically rather than as just a mechanical barrier.
6) Very effective as the L.E.D. button gets activated by the mere touch of the tongue or thumb.

Cons of this appliance

1) Patient compliance is required for the effectiveness of this appliance.
2) Low charge in the battery will hamper the treatment process.

Conclusion

In the present rapidly growing technological era, it has become imperative for dentists to provide hassle-free treatment in addition to the need to provide quality treatment to patients. This appliance outweighs conventional appliances by being more biocompatible, cost effective, easily adaptable and comfortable. Notably, this appliance serves as a reminder to the child rather than a punishment, thereby solving the prime need to help the child quit the thumb sucking habit.

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Chapter - 2
Nutrition and Oral Health

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Chapter - 2

Nutrition and Oral Health

Dr. Alankrita Chaudhary, Dr. Avneet Kaur and Dr. Nili Kumari

Abstract

Nutrition and oral health are intricately interconnected, as the food we consume plays a significant role in maintaining the health of our teeth and gums. A growing body of evidence demonstrates that an unhealthy diet high in added sugars, refined carbohydrates, and acidic beverages contributes to the development of dental caries. The frequent consumption of sugary snacks and beverages provides an ideal environment for oral bacteria to thrive and produce acids that erode tooth enamel, leading to cavities. Conversely, a diet rich in nutrient-dense foods such as fruits, vegetables, whole grains, lean proteins, and dairy products provides essential vitamins and minerals necessary for maintaining strong teeth and healthy gums. This chapter explores the relationship between nutrition and oral health, highlighting the impact of diet on dental caries, gum disease, and overall oral health. It also emphasizes the importance of a balanced diet, consisting of essential nutrients and avoiding harmful dietary practices, in promoting optimal oral health.

Keywords: Dental caries, health, nutrition, oral health

Introduction

Nutrition

The study of nutrition focuses on how food and nutrients affect our bodies and health. It entails comprehending the intricate relationships that exist between food, our bodies, and the environment. Maintaining physical health and preventing adverse oral health require proper nutrition. The nutrients required for optimum health can be obtained through a balanced diet that consists of a variety of meals from various food categories.

The way the body reacts to the food we eat is reflected in the oral cavity aswell. The adage that "the oral cavity is the mirror of the human body" is su pported by this [1].

The study of nutrition is divided into three areas: undernourished, over nourished, and malnourished. The primary concern of developing countries is malnutrition and under nutrition, whereas developed countries are concerned with over nutrition [2].

Macronutrients & micronutrients

These nutrients are divided into two categories: macronutrients and micronutrients. Carbohydrates, proteins, and fats are the macronutrients that make up the majority of the diet. Micronutrients, which contain vitamins and minerals, are required in minute amounts [2].

Macronutrients

The nutrients found in plants are essential in large levels as our bodies are unable to produce them on their own.

These macronutrients provide energy and support the body's many metabolic systems, growth, and development. The macronutrients include things like fats, proteins, carbohydrates, vitamins, and minerals.

Micronutrients

These are plant based nutrients, which are essential in very little quantities.

Micronutrients include vitamins, minerals.

Chapter Contents

- Introduction
- Micronutrients and macronutrients
- Relation between oral health and nutrition
- Therapeutic Diet

Relation between nutrition and oral health

Nutrition has direct impact on oral health [1], poor nutrition leads to adverse oral health like tooth decay, caries, gum diseases. Eating is an essential part of our living. Food we intake affects body in 2 ways:

- Systemic effects
- Local effects

Systemic effects are influenced by nutritional content and include the impact on general health, growth and development, cell renewal, tissue repair capacity, and disease resistance.

Food also have a local effect on tissues of body.

In dentistry, the interaction between food residues and oral bacteria resul ts in the majority of local physiological effects, which in turn cause plaque fo rmation and dental cavities.

There are classes of nutrients which have important effects on oral health [3]

- Carbohydrates and lipids-these provide energy.
- Tissue building proteins.
- Vitamins and minerals.
- Water comprising 55-65% of total body weight.

Depending on the amount of sugar they contain, carbohydrates are categorised as monosaccharides, disaccharides, or polysaccharides.

1. **Monosaccharides:** These are simple sugars that cannot be broken down further into smaller units. Examples include glucose, fructose, and galactose.

Disaccharides: These are formed by the combination of two monosaccharides. Examples include sucrose (glucose + fructose), lactose (glucose + galactose) and maltose (glucose + glucose).

Sugars are the most important elements in the development of caries. The most cariogenic sugar is sucrose, a disaccharide comprising glucose and fructose. When sucrose is consumed, it can contribute to the development of dental caries, which are cavities in the teeth. The bacteria in the mouth feed on sucrose and other fermentable carbohydrates, producing acids that can demineralize tooth enamel and lead to the formation of cavities. Over time, if left untreated, dental caries can cause tooth decay, pain, and even tooth loss. Therefore, a diet reduced in sugar should be used as a substitute [4].

Sugar substitutes
• Peanuts, walnuts, pea-cans, almonds, other type of nuts Popcorn, corn chips, potato chips, whole wheat biscuits, unsweetened dry cereals
• Cold cuts of meats (unsweetened)
• Sugarless chewing gums (Xylitol chewing gum- Xylitol has a similar sweetness to sugar. It prevents tooth decay, stimulates saliva production and also re-mineralises the enamel.)
• Poached Eggs
• Unsweetened fruit juices, freshly squeezed fruit juices
• Fresh fruits, salads

• Smoothies (Unsweetened)
• Baked potatoes, fried potatoes
• Vegetables such as carrot slices, celery sticks, cucumber slices
• Dates • Tofu
• Cubes of cheese

1. **Polysaccharides:** These are carbohydrates made up of many monosaccharides joined together. Examples include starch, glycogen and cellulose.
2. **Fat** is one of the macronutrients that provide the body with energy. Fat is a complex molecule made up of carbon, hydrogen, and oxygen atoms. It is an essential nutrient that is required for several bodily functions.
3. **Proteins** are large biological molecules composed of amino acid chains that serve a variety of functions in living organisms. They are necessary components of all living cells and play an important role in a variety of biological processes.
4. **Minerals** are necessary for the skeletal system, dentition, and the structural components of soft tissues.

- Minerals like Ca, Ph, Mg, K, Cl, Na, Fl, Zn, Fe, Cu, I and many more are significant.
- Ca, Ph, and Mg are crucial for tooth development.
- Fl improves resistance to cavities and is crucial for tooth development.

5. **Vitamins** are the group of organic compound which are essential for body.

- Vitamins can be Fat soluble like-vitamin A, D, E, K and water soluble like-vitamin C, B complex.

6. **Water** is a vital nutrient for the human body. It is required for many physiological functions and helps maintain overall health and well-being.

To ensure that the body can function effectively, these nutrients should be consumed in a balanced diet. Lack of nutrient intake can affect a person's ability to grow and develop, as well as their academic performance, susceptibility to repeated illnesses, and 0ral health [4].

A balanced diet contains	
Protein	10-15%
Fat	15-30%
Carbohydrates	45-65%

Our bodies get the energy they require to function from the food we eat. The body needs this energy for the synthesis of compounds, growth, and development, activity of the brain and nerves, and control over bodily processes. Decrease in the intake of nutritious food have a negative impact on oral health, growth and development [1].

Diets can be balanced or unbalanced, each with its own set of effects on the body. A balanced diet contains the appropriate ratios of all nutrient classes to prevent any deficiency, which could have a detrimental impact on oral health as well as the overall health of the body.

The impacts of essential nutrients on oral health [1]

Nutrients	Effects on oral health	Important sources
Fat	Dental caries	• Vegetable oils such as olive, canola, sunflower • Nuts • Seeds • fish
Protein	• Hypoplasia of deciduous teeth • Delayed eruption Rotated teeth, crowding, retarded growth of jaw bone	• Seafood • Low fat milk • Eggs • fish
Minerals		
Calcium	Improper tooth development	• Leafy Vegetables • Figs • Canned baked beans
Phosphorus	Improper tooth development	• Beans • Nuts • Fish • Dairy products
Magnesium	Improper tooth development	• Pumpkin seeds • Almonds • Peanuts • Soymilk • Oats
Fluoride	Improper tooth development, Dental Caries	• Fluoridated water • Grapes • Raisins

Selenium	Periodontal diseases	• Brazil nuts • Organ meat • Seafood
Vitamins		
Vitamin A	• Irregular dentin formation • Increased caries risk • Delayed eruption • Xerostomia • Increased risk of candidiasis	• Fish • Liver eggs • Dairy product
Vitamin D	• Incomplete mineralisation of teeth and bone • Delayed eruption • Malalignment of teeth • Enamel hypoplasia	• Flesh of fatty fish • Fish liver oil
Vitamin K	• Gingival bleeding • Candidiasis	• Broccoli • Cabbage • Spinach
Vitamin C	• Swollen gums • Loosening of teeth • Candidiasis	• Citrus fruits like orange, lemons • Bell pepper • strawberry
Vitamin B2	• Glossitis • Chelosis • Shiny red lips • Enlarged fungiform papillae	• Beef • Mushrooms • Almonds
Vitamin B4	• Angular chelosis • Mucositis • Stomatitis • Glossodynia • Glossitis	• Peanuts • Dried Grass • Pork brain and pork kidney
Vitamin B6	• Angular chelosis • Burning mouth • Glossitis	• Fish • Beef liver • Potatoes
Vitamin B12		• Bananas • Dairy products • Beef

Nutrition and periodontal disease

While local variables promote the development of periodontal disease in humans, it is thought that the speed of the destructive process is influenced to a large part by constitutional factors, nutritional status being one of them.

Periodontal disorders are characterised by the episodic and gradual breakdown of various tissues. The many host variables are vulnerable to

dietary impacts that effect on structure, repair, and defence on a systemic level. The epithelial barrier and attachment, periodontal ligament, gingival connective tissue, alveolar bone, cellular and humoral immune mechanisms, inflammatory response, gingival fluid composition, and crevicular epithelium (to invasion by antigenic or enzymatic irritants/toxins produced by bacteria) are the main targets in nutritional deficiency. All of these are vulnerable to nutritional deficiency.

Nutrition and oral cancer

In the genesis of oral and pharyngeal malignancies, nutrition is crucial.

Cancer of the head and neck is more likely to develop in those who are malnourished. Foods include both carcinogenesis's initiators and modifiers. The modifiers may have an impact on the carcinogen-metabolizing enzymes' activity, hormonal state, or immunological response.

Most chemical carcinogens need to be activated by enzymes. The mixed-function oxidase system, which is mostly accountable, is greatly impacted by the nutritional status and the quantities of certain nutrients, such as protein or fat consumption. These enzymes may be suppressed by certain dietary deficits, which would lower the body's ability to fight against chemical carcinogens.

Diets high in protein are likely to be high in calories, animal and other saturated fats, and calories that are linked to cancer. For optimal health and growth, one needs a minimum of 5% protein. An increased risk of mouth and pharynx cancer is linked to high consumption of saturated animal fats. Anemia or malnutrition lower the immune system's capacity to fight against cancerous cells.

Osteoporosis

Alveolar bone is vulnerable to osteoporosis since it helps to maintain the body's calcium balance. As compared to bone deposition, bone disease and resorption are somewhat more common in the elderly. The alveolar process is resorbed when teeth are lost because it can no longer perform its essential role of supporting teeth.

In order to increase the bone's resistance to mechanical and nutritional biochemical stresses, the elderly should supplement their diets (especially those of women) with calcium and vitamin D (to prevent the loss of alveolar bone), adequate polyunsaturated fats, and inexpensive proteins. They should also consume fewer calories, more vitamins C and B12, folic acid, iron, and other vitamin B family members.

Tooth erosion

Dental erosion is the process when tooth enamel is lost as a result of the acids in certain foods, drinks, or medications. Enamel, the hard outside coating of teeth, can erode to reveal the dentin layer below. Due to this, teeth may become sensitive, discoloured, or even develop cavities.

Tooth erosion can be due to extrinsic or intrinsic causes

Extrinsic	Intrinsic
• Dietary elements, such as eating and drinking acidic foods and beverages, such as citrus fruits, fruit juices, and carbonated drinks.	• Gastrointestinal issues that lead to stomach acid entering the mouth and wearing away tooth enamel, such as acid reflux or bulimia.
• Environmental elements include swimming pool chlorine exposure or over-brushing with a hard-bristled toothbrush.	• Increased mouth acid can result from dry mouth, which can be brought on by some drugs, medical problems, or lifestyle choices including smoking.
• Behavioural elements, such as clenching or grinding the teeth, which can eventually wear down the tooth enamel.	• Genetics, which can influence the durability of enamel and increase erosion risk in some persons.

Management of tooth erosion

Limiting acidic foods and drinks, practising proper oral hygiene, and scheduling routine dental check-ups and cleanings are all vital ways to stop tooth erosion. Dental bonding or veneers are used to repair the functionality of damaged teeth and fluoride treatments can also be used to strengthen your tooth enamel.

Fluoride: Fluoride is a mineral that has been used in dentistry for many years to help prevent tooth decay. Fluoride helps to strengthen the enamel on teeth, making them more resistant to acid attacks from bacteria and sugars in the mouth. It can be used in many ways in dentistry like: fluoride varnishes, fluoride tooth paste, fluoride supplements.

Therapeutic diet

Nutrition includes diet therapy for medical conditions. Therapeutic diets are typically unappealing. However, depending on the circumstance, they contain high or low concntrations of particular nutrients to satisfy the needs of the body.

Here are a few illustrations of therapeutic diets

- Increase your intake of calcium and vitamin D: These nutrients are essential for strong teeth and bones.

- **Eat plenty of fruits and vegetables:** These foods are high in fiber, which can help clean teeth and gums and vitamin C, which is important for gum health.
- High calcium and magnesium intake is advised during adolescence.
- Infants and young children have higher requirements for calcium, iron and protein.
- The needs for iron, calcium, magnesium, folic acid, and vitamins B6 and B12 rise during pregnancy and nursing.
- Elderly persons need to consume additional vitamins and minerals, such as chromium, zinc, and vitamin D.
- **Limit your intake of sugary and acidic foods:** Sugary and acidic foods can contribute to tooth decay and erosion.
- Limit your intake of sugary foods to 3-4 times a day when a structured meal plan is not being followed.
- Avoid eating or drinking anything with sugar or acid close to bedtime.
- Reduce your intake of sugary and acidic meals and beverages, and try to save them for mealtimes.
- Moms should only use formula milk, expressed breast milk, cow's milk, or water to fill a baby's bottle to avoid ECC.
- **Avoid hard and sticky foods:** These foods can chip or crack teeth or dislodge fillings. Examples include hard candy, popcorn kernels, and chewing gum.

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Chapter - 3
COVID-19: Oral Manifestations

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Chapter - 3

COVID-19: Oral Manifestations

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Abstract

Worldwide, the human population has been impacted by the coronavirus-19, which has caused all age group's lives to sluggishly progress and cost everyone two priceless years in 2020 and 2021. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a single-chain RNA virus that is the cause of novel coronavirus disease known as COVID-19. Within a short period of time, COVID-19 has evolved into a global pandemic that affects people of all ages and has a significant mortality rate, a wide range of aftereffects in survivors, and a negative socioeconomic impact on society. Coronavirus disease not only affects the respiratory system of an individual but also has an early-stage effect on the oral cavity. Normally, this oral cavity manifestation goes unnoticed, but if it is, it can greatly aid in the early detection and treatment of COVID-19 before it becomes severe in many people.

Dysgeusia is the first oral symptom and most prevalent oral cavity manifestation in people with coronavirus disease. Other symptoms include salivary gland dysfunction, xerostomia, and oral mucosal lesions. Because the majority of these manifestations can be observed during the asymptomatic phase of the coronavirus illness course, these indications can be used to aid in the prompt diagnosis of this disease. Thus, it helps in minimizing the transmission rate of COVID-19 between the individuals as quarantine and treatment measures can be started at the initial stage.

Keywords: COVID-19, Dysgeusia, oral lesions, xerostomia, ulcers

Introduction

Beginning in December 2019, the corona virus disease (COVID-19) has been wreaking havoc over most of the globe. Every age group is exposed to and afflicted by the corona virus, which disrupts everyone's normal way of life. The severity of the disease, its treatment regimen, its after effects, and its mortality rate, together with its symptoms, have all had a devastating impact

on humanity. The highly virulent virus that has quickly spread over the world presents a significant problem for the healthcare industry. Covid 19 patients have both pulmonary and extrapulmonary symptoms documented.

The oral cavity always allows for early diagnosis and provides a good picture of the prognosis of numerous disorders [1]. Saliva has been implicated in the transmission and diagnosis of Coronavirus illness [2, 3, 4, 5, 6]. Various alterations within the oral cavity have been suggested as non-invasive diagnostic criteria, providing an indication of the severity of the illness. Changes in the lips, tongue, palate, gingiva and buccal mucosa were observed, with gustatory impairment being the most common. Other oral lesions observed were ulcers, petechiae, small blisters, white and erythematous plaques, and gingivitis. These oral lesions appeared prior to or at the same time as the onset of respiratory symptoms. As a result, oral mucosal lesions were more likely to emerge as co-infections and secondary symptoms [7].

Oral manifestations of Covid-19

Ulcer, erosion, vesicle, pustule, fissured tongue, macule, papule, pigmentation, halitosis, white patches, haemorrhagic crust, necrosis, petechiae, erythema, swelling were among the oral signs. The most prevalent characteristics that predict the severity of oral lesions in patients with COVID-19 appear to be older age and the severity of the disease.

The following are the oral manifestations of COVID-19:-

Loss of taste

Covid-19 patients present with early symptoms of cough, fever, myalgia, and taste impairment. It was observed that people who had contracted covid 19 infection had loss of taste or alteration of taste or smell, and had positive early symptom or only clinical symptom present. Sometimes the patient's only symptom is a loss of taste and smell. The most prevalent signs of the condition are cough and muscle and joint discomfort. Other symptoms such as dry mouth, anosmia and dysgeusia may also be present. COVID 19 symptoms include runny nose, change in taste, and facial pain [8].

Dysgeusia and dry mouth can manifest in most patients within 5 days of aquiring a COVID-19 infection and it typically lasts for 2 weeks, or up to 4 weeks in more severe cases. Taste loss is caused primarily by plaque and a swollen palate, rather than by alterations in the taste buds. Smell related changes is mainly due to the infection because of the viral load in the upper respiratory tract [1].

Geographic tongue

The geographic tongue associated with COVID-19 is an inflammatory condition that often affects the tongue's lateral and dorsal surfaces [9]. This COVID tongue has irregular white borders and what appear to be bald and red regions of varying widths. Emotional stress, habits, systemic conditions can be some of the factors which may be responsible for COVID tongue.

Dry mouth/Xerostomia

One of the first indications of COVID-19 before the signs and symptoms in the upper respiratory tract may be dry mouth. When it comes to early identification and treatment of COVID patients, this symptom can be quite helpful. Freni *et al*. described the symptoms of xerostomia, which began with the other COVID-19-related symptoms, in 32% of individuals who had the condition. Xerostomia was evident only in 2% of the patients when the COVID-19 symptoms vanished [10]. Another study reported xerostomia to occur before other symptoms in the disease sequele [11]. In COVID patients, the impact of the virus on the salivary glands may be a factor in the development of xerostomia. Stress, anxiety, and hospitalization during the coronavirus sickness could be additional factors that contribute to this [12].

Taste is definitely considered to be a good stimulant for saliva secretion. In their cohort study, Bidasee *et al*. revealed that 50% of the patients had both dysgeusia and xerostomia, demonstrating its co-occurrence [8]. According to other studies, patients with a runny nose and congestion may mouth breathe, which might exacerbate their dry mouth [13, 14]. However, neither xerostomia nor dysgeusia were shown to significantly correlate with either nasal congestion or rhinorrhea, according to Bidasee *et al*. [8] In COVID-19 patients, bitter taste and dry mouth were found to be correlated, according to Luo *et al*. [15].

Another reason is that a loss of smell can result in a lack of indirect stimulation of saliva. In their study of three cases of parotitis in COVID patients, Lechien *et al*., suggested lymphadenitis in the parotid gland as a cause of xerostomia, where there is an increase in parotid gland secretion, resulting in a blockage of the Stensen duct, saliva retention, and dry mouth due to hyposalivation [16].

Another cause of xerostomia is the high number of medications given to COVID patients, which include antiviral treatments such as remdesivir, ritonavir, lopinavir, hydroxychloroquine and interferons [17]. Other probable reasons for xerostomia in COVID-19 individuals include zinc insufficiency, viral neurotransmission, salivary gland inflammation and ACE2 expression [18, 19, 20, 21].

Oral lesions, ulcers and blisters

Oral mucosal lesions have developed as primary symptoms in a few cases and as subsequent symptoms in a few others. Appearance of oral lesions was simultaneously found with loss of taste and smell in few patients and more severe oral lesions were reported in older patients and in severe COVID-19 infection.

Aphthous like ulcers appeared as single or multiple shallow ulcers with erythematous halos and yellow-white pseudo membranes seen on keratinized and nonkeratinized mucosae with latency time between 2 and 10 days. Stress and immunosuppression secondary to COVID-19 infection or increased level of tumour necrosis factor (TNF)-α in COVID-19 patients with chemotaxis of neutrophils to mucosa may lead to aphthous-like lesions in COVID-19 patients.

Ulcerative and erosive painful lesions with irregular borders can be seen on hard palate, tongue and labial mucosa. Various factors including drug eruption, vasculitis, or thrombotic vasculopathy secondary to COVID-19 may be attributed as various causes for development of ulcerative and erosive lesions.

Other lesions like vesicles, pustules, necrotising periodontal disease were seen in patients with covid 19 infection. Petechiaes on the lower lip, palate and oropharynx mucosa due to thrombocytopenia in covid 19 patients or due to prescribed medications were present. When these manifestations arise as primary symptoms, they can aid in the primary diagnosis. However, they frequently go unrecognized due to a lack or delay in intraoral inspection during a clinic or hospital visit. Angela *et al*., described three cases of herpetic recurrent stomatitis-like ulcerations and blisters on the palate and tongue. One of these three instances had blisters on the labial mucosa and desquamative gingivitis. All of these cases were treated with topical antiseptics, according to reports [22].

Amorim Dos Santos *et al*., demonstrated that COVID-19 could potentially contribute to severe oral health outcomes such as opportunistic fungal infections, recurrent oral herpes simplex virus infection, and gingivitis as a result of a compromised immune system or medications given for COVID-19 [9].

Oral candidiasis

The COVID-19 virus affects the immune system, which leads to an increase in opportunistic infections, which typically manifest in the oral

cavity. Oral candidiasis has been reported in patients with severe COVID-19, particularly in those with underlying medical comorbidities and those taking antibiotics. Cases of oral candidiasis in COVID patients were reported by Abanoub Riad *et al*. The affected areas with white membranous patches were the dorsal aspect of the tongue, soft palate, floor of the mouth, buccal mucosa, and oropharynx. These patients were treated with topical antifungals and local antibacterial mouthwash, according to reports [23]. Petechiae and gingival melanin darkening are two other linked oral cavity abnormalities in COVID patients.

Other manifestations

Covid-19 virus infection can induce sialadenitis of the submandibular salivary glands as well as inflammation of the parotid glands. In a case report by Capaccio *et al*., a 26-year-old man developed painful parotid gland swelling and the ultrasonography revealed enlarged and diffuse hypoechoic parotid gland [24, 25].

Corona virus primarily infects the sulcular and periodontal pocket epithelia and has a detrimental impact on periodontal tissues. Prevotella intermedia is frequently found in COVID-19 patients and Coronavirus may predispose individuals to periodontal disease by bacterial coinfection caused by Prevotella intermedia. Patel *et al*. found halitosis, severe gingival discomfort and gingival sulcus hemorrhage in a patient suspected of having COVID-19 [26].

Oral manifestations of Mucormycosis in Post COVID-19 patients

Oral mucormycosis manifests as white fungal patches on the tongue, ulcers, and gingivitis. Mucormycosis patients may also experience toothache, tooth loosening, and jaw involvement. These oral symptoms may be attributable to a weakened immune response found in COVID-19 patients or patients who need steroids during COVID treatment. Dental symptoms of mucormycosis can occur after COVID, in long-term diabetics, in individuals with a poor immune response, and in those with poor dental hygiene.

Conclusion

The oral cavity is crucial because it is one of the main entry points for bacteria, viruses, and fungi that can harm a person's overall health. In a person with COVID-19, oral symptoms might be a primary or secondary symptom. These symptoms in the oral cavity should be highlighted and treated with care. Poor general and oral hygiene can contribute to viral load, which can provide the right environment for COVID-19 to develop into a severe form. The

elevated viral load is strongly related to poor oral hygiene and other factors that affect the oral cavity such as old age and severity of infection, opportunistic infections, stress diseases like diabetes mellitus, immunosuppression other systemic diseases may predispose to development of oral lesions. Both the systemic and oral signs will be lessened by lowering this virus load. Keeping up a rigorous oral hygiene routine might thereby lessen the severity and morbidity of COVID-19.

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Chapter - 4
Oral Biopsy-Methods and its Applications

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Chapter - 4

Oral Biopsy-Methods and its Applications

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Abstract

Biopsy is the removal of tissue from a living person for microscopic examination to confirm or to establish the diagnosis of a disease. The purpose of this chapter is to review the indications and contraindications of biopsy. Various types of biopsies, the mode of anesthesia and instruments required for the particular type biopsy of the lesion. It is also very important to know the essential biopsy principles and how to choose a particular type of biopsy. One should be familiar with the fixation of the tissue and the transport of the tissue specimen to be carried out. Similarly it is also important to be acquainted with the possible reasons of failure of histological diagnosis. Therefore the meticulous forehand planning prior to performing biopsy is necessary for the precise biopsy outcomes.

Keywords: Brush biopsy, incisional biopsy, excisional biopsy, punch biopsy

The diagnostic sequence

- Recognition of deviation from normal.
- History.
- Methodical examination of the oral cavity.
- Reexamination of the apparent alteration from the normal.
- List of possible diagnoses.
- Development of differential diagnosis.
- Development of working diagnosis.
- Para-clinical findings like radiographs, biopsy, microbiology, blood count etc.
- Formation of the definitive diagnosis supported by Para clinical findings.

Introduction

Synonym-for Biopsy is “Tissue sampling”.

Word “Biopsy” is Greek word meaning “view of the living’. It is an important diagnostic tool. The bacteriologic examination of pus, bodily fluids etc. are not included in biopsy. Cytological examination of smears can be considered a special type of biopsy.

Definition

According to Peter A Reichart Hars, Peter Philipsen (2000)

The biopsy can be defined as, “a diagnostic procedure, whereby tissue or cellular material is removed from the living organism in order to be microscopically examined and to prepare a description of the histological findings”.

Indications for biopsy

1. There is a general rule is-“When in doubt, BIOPSY”.
2. In case of a great thought of being malignant.
3. Ulceration that persist for more than 3 weeks and fail to heal.
4. Long standing observable or palpable swelling in the vicinity of comparatively normal area without any clear diagnosis.
5. Persistent white lesion of the oral mucosa.
6. Lesion not responding within 10-14 days of local management protocol.
7. Bony lesions not specifically identified by clinico-radiographic methods.
8. Lesion persisting for greater than two weeks without any apparent etiology.
9. Lesions interfering with reginal function like fibroma.
10. In diagnosis of systemic diseases.
11. Lesion having features of being malignant.

Contraindications for general practitioner

There is no absolute contraindications but

- If patient’s medical history reveals any blood dyscrasias or if the patient is undergoing anticoagulant therapy.
- Pigmented lesion. Leukoplakia of floor of mouth with pigmented lesion.

- Biopsy of malignant tumors should be carried out by taking into consideration the danger of metastasis.
- Biopsy of malignant tumors should be done by taking into consideration the danger of metastasis.
- Intra-bony lesions with internal structure radiolucent should be biopsied only after diagnostic aspiration.
- Vascular lesions should be biopsied only after diagnostic aspiration.

Essential biopsy principles

- Fill an informed written consent form mentioning the biopsy indication with details of probable risks towards the procedures.
- Surface antiseptics with Iodine content not to be used, since may have a propensity to permanently stain certain cells of the tissues.
- The type of anesthetic used & whether tissue was removed with the scalpel or with the electrocautery should be mentioned.
- Inject the anesthetic solution away from the biopsy site never into the biopsy site.
- Choose most suspicious areas, include adjacent normal tissue in the biopsy and avoid sloughs or necrotic areas. Multiple areas may need to be sampled for large lesions with inclusion of each and every piece to be subjected for the examination.
- The size of the specimen has to be minimum 1 X 0.6 cm X 2 mm deep and suture the specimen to control.
- Always aspirate bony cavities before opening into them.
- The suction should be kept away from the surgical site when the tissue is ready to be removed so as to avoid sucking up the specimen.
- The specimen bottle should be labelled with name, age, sex of the patient including the date and time of biopsy. Write pervious biopsy number if any.
- The type of biopsy, the site from where it is obtained and nature of tissue should be mentioned.
- Mention the brief clinical history, history of adverse and parafunctional habits, radiological and other relevant features if any with tentative clinical diagnosis.

- Check that the report is corelating the clinical diagnosis and investigations. Discuss with the pathologists or repeat biopsy if diagnosis is unclear or not understood.

Mode of anaestesia

- Local anesthetic to be given deep or in a area in the vicinity of the biopsy site and not in to the site itself.
- A block may also be given provided the effect of adrenaline within the anesthetic solution is not lost.

Instruments

- Fine surgical forceps, vessel clamps, needle holder, suture scissors sutures Lagenbeck retractors, gauze packs.

Forms of biopsy

- Excisional
- Incisional
- Punch
- Fine needle aspiration
- Needle/core
- Aspiration
- Guided biopsy techniques
- Cytology

Excisional biopsy

- If the pathologically altered mucosa is small, the whole lesion including a rim of adjacent normal tissue is removed in toto. This type of biopsy is referred to as an excisional biopsy.
- Excisional biopsy is can work as a diagnostic procedure and treatment.

Indications

- Surgically reasonably accessible.
- Benign and less than 2-2.5cm in diameter.
- May be “shelled out”.
- Small, well defined, bony lesions.

Incisional biopsy

In an incisional biopsy, a small representative part is removed from the boundary zone where normal and pathologically altered tissue adjoin.

Indications of incisional biopsy

- Lesion larger than 1.5-2.0cm in diameter and a single biopsy cannot encompass all of the characteristics of the change.
- Large intrabony or soft tissue lesions.
- Diffuse lesions.
- Suspected malignancies.

Procedure

- Vertical incision through the lesion with the creation of a wedge shaped tissue section that includes adjacent normal mucosa.
- Both connective tissue and epithelium should be included.

Review of literature

According to study by Kinsukawa J *et al.* (2000): Demonstrated that cytokeratin was present in the peripheral blood of two out of ten patients 15 minutes after the incisional biopsy of an oral squamous cell carcinoma, there by demonstrating that there was dissemination of cancer cells which result in metastasis and suggested that chemotherapeutic drugs should be administered prior to biopsy to minimize risk of metastasis in such patients.

- The low occurrence of blood borne metastasis, but this area needs further investigations.

Punch biopsy

- This type of biopsy is an alternative to an incisional biopsy. This is a form of biopsy is best suited for diagnosis of mucosal abnormalities requiring several biopsies. It produces clear sharp incision.

Procedure

- Place punch at intended site with light pressure and rotational movement, surgical core is created and is released at its base with scissors.
- Multiple plugs can be taken
- Cheeks, lips and accessible regions can be biopsied using punch.
- Palatal & gingival areas do not allow adequate biopsies with punch.

Vital staining for selecting most appropriate area

Toluidine blue staining

- Apply 1% aqueous solution of toluidine blue stain.
- Rinse with water.
- Decolorize with 1% acetic acid.
- Rinse with water and see for any binding.

Handling the biopsy

The obtained tissue is very small and bleeding surface is placed down on a stiff piece of paper.

Reported complication

Moule I, Parsons PA *et al.* (1995) reported a case of surgical emphysema that resulted after performing a punch biopsy intraorally because the patient sneezed just after the procedure.

Fine needle biopsy

This type of biopsy may be used in the deep-seated lesions to remove a very small piece of cells from a deep-seated lesion (FNAB).

Aspiration cytology gun with syringe & needle

Indications of fine needle biopsy

1. A suspected tumor of salivary gland.
2. Enlarged lymph nodes in the submandibular or submental region.

Procedure

- A 23 gauge needle attached to a10cc disposable syringe & several, sharp, quick, jabbing strokes are made with needle at different angles.

Disadvantages

- Experienced pathologist is essential for interpretation.
- Small sample may be un-representative.
- Definitive/final diagnosis may not always be available**.**

Needle/Core biopsy

- Needle/core biopsy is an alternative to FNAB.
- Up to 2 mm diameter needle is used to remove a tissue core.
- Processing of the specimen is similar to a surgical biopsy.

Advantages

- Use of large sized sample than fine needle aspiration conserves the architecture of the tissue specimen.
- Definitive diagnosis more likely than FNA.
- Useful for inaccessible tumors like pharynx.
- Aspiration of a centrally located radiolucent lesion in the bone is used to differentiate cavities filled with fluid, vascular lesions, hematomas, sialocele and empty cavities.

Disadvantages

- Danger of seeding some form of tumor into the tissue.
- Chance of anatomical structure in the vicinity getting damaged.

Guided Biopsy Technique

- Used in many areas of body primarily as a method of obtaining material for microbiological examination that would otherwise only be available by open operation.
- Used in situations where simple percutaneous fine needle biopsy is possible but cannot be relied upon to sample a questionable area and can be (1) USG guided (2) CT guided.

Ultrasonically guided biopsy technique

- Particularly suited for relatively superficial regions.
- Gives real time image.
- Has ability to image needle tip as it is guided into the target lesion.
- “Ecogenic tip” needles are available.
- Disadvantage is that the needle tip is more difficult to define with USG than with CT.

CT guided biopsy technique

- Enable a two dimensional image with skin markers to be produced, from which the entry point, angulations and depth of insertion of the biopsy device may be electronically planned.
- The disadvantage is that, as the biopsy device is advanced towards the lesion, the procedure must be interrupted, and repeat scanning is performed, at intervals which depend upon the anatomic complexity.

Cytology

- **Definition:** "It is an art and science of interpretation of cells from human body that either exfoliate (desquamate) freely from the epithelial surface or are removed from various tissue sources by various clinical procedures".
- Two types-Exfoliative cytology and Oral brush cytology.

Intraoral exfoliative cytology

- Originally developed by Silverman and Sandler.
- In this technique cells scrapped from the surface of a lesion or occasionally of material retrieved from the cyst aspirate is examined.

Indications

- For rapid laboratory evaluation of premalignant or malignant lesion.
- Sequential assessment of mucosa that has been previously dealt by excision or radiation to remove a malignancy.
- Evaluation of vesiculo-bullous lesion where facilities of Tzanck smear is not available.

Cytological smear technique

- Material is scrapped off the mucosal surface with spatula.
- Collected material spread evenly onto a glass slide & immediately fixed with 70% alcohol.
- Spray fixation is also possible.

Tzanck test

- Useful in diagnosis of vesiculo-bullous lesion and viral eruptions.
- Top of early lesion is removed.

- Floor is scrapped off.
- Thin smear is prepared, air dried, can be fixed.
- Stained with Wright stain, Giemsa stain, or H and E.

Standard classification used in oral cytoloy reports

- **Class I:** Normal cells.
- **Class II:** Some atypical cells, but no evidence of malignancy.
- **Class III:** Changes in nuclear pattern of intermediate nature; no evidence of malignancy, but clearly aberrant cells are present.
- **Class IV:** Suggestive of malignancy.
- **Class V:** Obvious malignancy.
- **Note:** A report of CLASS III, IV and V changes should always be followed by a biopsy of the lesion.

Oral brush cytology

- A brush is used to take a sample of cells from epithelium layers-basal, intermediate and superficial.
- Brush is firmly rotated over a lesion.
- Collected cells transferred to a slide.

Review of literature

- Sciubb JJ; JIDA 1999, a study from United States-claims a high sensitivity and specificity to detect dysplasia.

Scissors biopsy

- It is removal of skin tissue for a biopsy specimen in one of the ways.

Shave Biopsy

- It involves scraping of the lesion either superficially or deeply using a scalpel or a razor.

Fixative

- 10% Formalin solution is used which fixes specimen through forming intermolecular bridges proteins and there is formation of cross links between protein end groups.
- The amount of fixative-10 to 15 times greater in volume than the tissue specimen.
- Plastic containers with screw caps & with an outer vial.

Possible reasons for failure of histological diagnosis

- Poorly fixed or damaged specimen at the time of removal.
- An un-representative specimen is of the lesion or it is very small.
- The critical features of the lesions are not included in the plane of histological section.
- The condition having non-diagnostic histological characteristics. E.g.; aphthous ulcers.
- The histology features having numerous likely causes. E.g.; granulomas
- The features of histology that are hard to interpret like sometimes may be so poorly differentiated malignant tumors that determination of their type is difficult.
- Inflammation present may mislead the diagnosis.

Conclusions

- After doing a thorough clinical examination for reaching to the final diagnosis, from the working or the provisional diagnosis biopsy is the gold standard which is required to be advised. When posting a case for biopsy, minute future planning and executing the same carefully can significantly upgrade the diagnostic value.
- Moreover, carefully handling the tissue specimen with timely and suitable fixation will lead to a confident histological diagnosis and will avoid unnecessary repeat procedures.

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ARTIFICIAL INTELLIGENCE AND EMERGING TECHNOLOGIES

PINKAL JAIN | PRATEEK GUPTA

Artificial Intelligence and Emerging Technologies

IIP Series

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AN INSIGHT INTO ARTIFICIAL INTELLIGENCE AND EMERGING TECHNOLOGIES IN DENTISTRY

Abstract

The field of dentistry, like many other sectors, is experiencing a transformation from emerging technologies, notably artificial intelligence (AI). This paper provides an overview of AI and other cutting-edge technologies currently influencing dentistry. AI has found applications in diagnosis, decision-making, and treatment planning in oral radiology, orthodontics, periodontics, and surgical procedures. In addition to AI, other technologies such as intraoral scanners, CAD-CAM systems, 3D printing, CRISPR gene editing, teledentistry, virtual reality, imaging techniques, Diagnodent, and dental lasers are shaping the landscape of dental practice. These technologies promise improved efficiency and enhanced patient care. However, challenges related to cost and implementation remain. Training for clinicians and staff to effectively utilize these technologies is imperative for their successful integration into dental practice. Overall, it is evident that AI and emerging technologies are poised to revolutionize the field of dentistry, offering innovative solutions to existing challenges and paving the way for the future of dental care.

Keywords: Artificial Intelligence, emerging technologies.

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Technology is leading the world in current times and has penetrated every field easing people's lives. Technology is revolutionizing healthcare and the way medicine is practiced. Likewise, dentistry is influenced and benefited by current technologies including artificial intelligence. Technology means the practical and industrial use of scientific discoveries.[1] Technology introduces means and methods that make the job easier manifold, save time, and be cost-effective, thus making the lives of patients and healthcare providers much easy and more comfortable. Artificial intelligence (AI) is an emerging technology in healthcare and has given much more emphasis to the role it plays in diagnosis, decision-making, and treatment. Artificial intelligence is computer systems that mimic human intelligence and perform tasks that normally require human intelligence.

Current and emerging technologies in dentistry are artificial intelligence, intraoral scanners, CAD-CAM, imaging techniques, virtual reality, teledentistry, 3D printing, etc. This chapter summarizes these technologies that are impacting dentistry.

I. ARTIFICIAL INTELLIGENCE IN DENTISTRY

The history of artificial intelligence started with the Turing test introduced by Alan Turing in 1950, if a machine can trick a human being into thinking that it's human, then the machine is said to be intelligent. Since then, AI has been successfully experimented with in various domains, including medicine and a little later dentistry. AI plays a main role in diagnosis and decision-making in dentistry. In oral radiology, AI has been used in the diagnosis of dental caries, sinusitis, osteoporosis, risk assessment of oral cancer, implant planning, implant detection, detecting and classifying odontogenic cysts, and tumours from intraoral radiographs. AI is also used in differential diagnosis, differentiating ameloblastoma, odontogenic keratocyst, and metastasis from non-metastatic carcinoma from computed tomography (CT) images. AI has also been used in the staging of oral cancer which helps in preventive measures to avoid the progression of the disease.[2,3] Early diagnosis plays a huge role in preventive dentistry and patient education programs. To achieve these diagnoses convolutional neural networks that are a deep learning method are implemented and the algorithms are trained with radiographic images.

Deep learning a subset of AI, is capable of assessing the need for orthodontic extraction. Automatic customized tracing and cephalometric analysis with AI technology help in planning orthodontic treatment, AI can also help in treatment outcome analysis. AI-based orthodontic monitoring will allow orthodontists to evaluate the progress of treatment from afar through patient phones and unique tools.[4] Invisible aligners in orthodontics are a revolutionary alternative to conventional metal brackets and have changed the way malocclusion is treated. Invisalign has addressed the concerns of aesthetics, periodontal maintenance, discomfort, etc associated with metal brackets. Apart from comfort and aesthetics, Invisalign also reduces treatment duration, yet the cost and achieving precise occlusion is a concern.[5,6] Patient's virtual models i.e. impressions and records are shared with the laboratory. The orthodontist receives the patient's virtual 3-D models in which the treatment plan is furnished for tooth movements and the orthodontist can visualize virtual corrections of each stage and if any corrections are required can be suggested to the lab. Once the orthodontist approves, aligners are fabricated. The software produces a series of aligners that will correct malocclusion. This entire process is done through AI-based software.[7]

AI algorithms can accurately detect and interpret alveolar bone level and radiographic bone loss and can be a useful adjunct to periodontal diagnosis and treatment planning.[8] AI algorithms can predict and diagnose periodontally weak teeth and assess the need for tooth extraction. These AI algorithms can be a valuable adjunct in periodontal maintenance and preventive measures. AI is a useful tool in the virtual planning of surgeries in implant placement, reconstruction of facial defects, and orthognathic surgery. AI software will allow clinicians to virtually perform surgery and they can anticipate and predict the course and outcome of surgery.[9]

A dental inspection software (smart Margin and scan clarity, Pearl) can analyze intraoral scans and give feedback on prepared teeth. Laboratories can also use this software to assess the preparations. Smile designing software can assist in virtual planning, patient education, and outcome analysis. This AI software proposes tooth shape and alignment, this design can create a mock-up, preparation, or surgical guide.[4]

AI assists in the maintenance of dental records, and could also assess smoking status from electronic records. AI systems could assess and diagnose temporomandibular disorders. AI could predict patients' age from radiographs and facial reconstruction with machine learning is a valuable tool in forensic dentistry. AI helps in preventing fraud claims with dental insurance. It is expected in the near future dental clinics will adopt AI comprehensive care system, patients' histories and records managed by AI systems will also help to understand oral diseases better and improved patient care. AI will minimize human error by providing comprehensive feedback regarding patients' medical history, allergic history, disease, and drug interactions, overall improving treatment outcomes and patient care.[4]

II. INTRAORAL SCANNERS

With the use of intraoral scanners, tedious impression and model preparation techniques are replaced saving clinician time and improving the accuracy of tissue registration. Intraoral scanners (IOS) digitally reproduce the 3-dimensional (3D) geometry of the intraoral tissues. scanners project a light source/laser on the surfaces being scanned and the sensors capture video or images, these images are processed by software to produce a 3D model. Multiple brands and generations of IOS systems and technologies are available, that can be chosen based on applications like orthodontics, restorations, fixed partial dentures, implant surgical guides, etc.[10]

1. CAD-CAM

CAD-CAM stands for computer-aided design and computer-aided manufacturing. The system consists of three components, a scanner that transforms geometry into digital data, software that processes this data into a design/product, and a third manufacturing unit/ milling machine that converts the design into a product. CAD-CAD unit can be chairside or at the laboratory. CAD-CAM restorations are fabricated by a subtractive technology that has superior properties.[11] These systems replace conventional tedious techniques of restoration, dentures, and prostheses fabrication, saving clinician time with added accuracy and patient satisfaction. Disadvantages associated with CAD-CAM systems are the associated expenses, the need for knowledge of technology and sensitive operations, maintenance, and the wastage of restorative material. Some of the currently available CAD-CAM systems are CEREC AC

(Sirona, Charlotte, NC, USA), E4D Dentist (D4D Technologies, Richardson, TX, USA), iTero (Cadent, Carlstadt, NJ, USA), and Lava COS (3M ESPE, St Paul, MN, USA). Davidowitz, G., & Kotick, P. G. (2011).[12]

2. 3D Printing

3D printing is additive manufacturing technology introduced in 1986 by Charles Hull. It is a three-dimensional printing technology based on CAD models using standardized materials to create 3D objects in an automated process. The advantages of this technology include high precision and rapid production. There are three 3D printing technologies, powder bed fusion, light curing, and fused deposition modeling. In dentistry, this technology is used in fabricating crowns and bridges, dentures, splints, working models, and surgical implants and guides. 3D printing brings about a means of fabricating complex models with more efficiency, and less wastage of material. However, the system has challenges of high cost and knowledge of the operation. Yet this technology is going to override conventional means and be the future of dentistry.[13]

3. CRISPR

This acronym stands for Clustered Regularly Interspaced Palindromic Repeats. CRISPR is a programmable protein that can edit, eliminate, and turn on/off the genome. One of the gene editing technologies with high efficiency and accuracy is promising in cancer therapy by introducing gene modifications in cell lines, organs, and animals. This technology is playing a vital role in preventive and predictive therapy in dentistry. Other applications in dentistry include the prevention of dental caries, dental plaque formation, treat salivary dysfunctions in Sjogren's syndrome, tooth and palate development, and TMJ disorders.[14,15]

4. Teledentistry

Teledentistry is telehealth using a combination of dentistry and telecommunications, it involves the exchange of clinical information and relevant imaging over a remote distance for the purpose of consultation and treatment planning. For urgent dental care, it is encouraged to assess risk, triage, and manage remotely over a telephone or video to provide urgent dental care. This unique way of healthcare delivery brings ease of use, patient satisfaction, and increased access to dental services providing specialist care, minimizing time of work, and minimizing travel for consultations. This helps the dentist and patient to save time on the first visit and be well prepared for the second visit. This can be valuable in this digital era and hectic and busy lifestyle.[16] There are still certain issues to be addressed before teledentistry becomes a routine thing but can be of immense benefit to society with limited dentists and inaccessibility.

5. Virtual Reality

Virtual reality is a computer-generated three-dimensional world in which the users interact with virtual objects. Augmented Reality is another technology that superimposes a computer-created virtual scenario over an existing reality to create a perception through the ability to interact with it. This can be made into applications in mobile devices to blend digital components into the real world in a way that they enhance each other. In dentistry, its

primarily used in the field of dental education from skill training in tooth preparation to complex maxillofacial rehabilitation approaches. This simulation exercise enhances student dexterity skills and the use of 3D vision in virtual learning improves the performance of the students. Other simulation exercises in dentistry include caries excavation, nerve block, reconstruction of facial defects, and learning 3D anatomy. This technology is also helpful in allaying dental anxiety especially in pediatric cases distracting them to reduce the emotional response.[17]

6. Imaging

Cone beam computed tomography (CBCT) imaging technique based on a cone-shaped X-ray beam centered on a 2-D detector. It does one rotation around the object and produces a series of 2-D images that are re-constructed into 3-D images. This technology was developed in 1984 by Aboudara et al.[18] CBCT is the new way of imaging in dentistry with reduced radiation dose, reduced exposure time, and compatibility with surgical, prosthetic, aesthetic, and orthodontic analysis and design software and it makes the ideal imaging technique in dentistry.[19] CBCT is used in oral surgery for surgical assessment and planning for impacted teeth, cysts, tumors, implant and orthognathic surgeries, and diagnosis of inflammatory conditions and fractures of the jaws and the sinuses. CBCT can be used in pre- and post-dental implant placement assessment.[18]

7. Diagnodent

Diagnodent is a state-of-the-art device used to detect early dental caries. It is a portable device that uses sound pulse and laser to detect caries earlier in comparison to conventional than traditional methods. The accuracy of detection being higher is helpful in early preventive measures.[20]

8. Dental Lasers

LASER means “Light Amplification by Stimulated Emission of Radiation” which generates electromagnetic radiation of uniform wavelength, producing coherent, monochromatic, intense, and collimated light.[21] Lasers have a wide array of uses in dentistry in periodontal surgeries, dental implants, caries prevention, caries removal, and cavity preparation, photobiomodulation (PBM) in dentistry, laser-supported endodontic treatments, fluorescence-supported oral cancer diagnosis, and treatment, lasers in wound healing, laser-driven biopsies. Lasers are being increasingly used in cancer healing and other domains of dentistry and are promised to be a reliable mode of treatment.[22]

CONCLUSION

Artificial intelligence and other emerging technologies in dentistry are a boon to the field as they improve operator efficiency and improved patient care. Yet the cost and implementation are a concern. Clinicians and assistants need to be trained to adopt and use these technologies to the best. It appears that AI and technologies are going to be the future of medical and dental practice.

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ARTIFICIAL INTELLIGENCE IN PROSTHODONTICS

Abstract

Artificial intelligence (AI) encompasses a broad spectrum of emerging technologies that continue to influence daily life. The evolution of AI makes the analysis of big data possible, which provides reliable information and improves the decision-making process. This chapter focuses on the specific application of AI in Prosthodontics, emphasizing its role in digital impressions, fixed prosthodontics, and implant dentistry.

Here in this chapter, capabilities of AI in accurate diagnosis, prognostic evaluations, and creative prosthetic construction are emphasised. The technology's contributions extend to implant recognition, categorization, and error reduction in dental procedures, enhancing overall treatment techniques. This chapter also includes AI's involvement in Maxillofacial Prostheses, showcasing its ability to create customized prosthetics based on anthropological calculations and patient preferences. The discussion expands to AI's impact on vision impairment, introducing smart reading glasses and bionic eyes as examples of how AI improves the lives of those with visual impairments.

The chapter is concluded by addressing the current state of AI in healthcare, acknowledging its limitations and projecting a visionary outlook for the next 5-10 years. Anticipated advancements include improved algorithms, data integration, and collaborative efforts between AI and human expertise for precision therapies.

Keywords: Artificial Intelligence, Prosthodontics, Maxillofacial Prostheses, AI in Healthcare, and Anticipated Advancements.

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In the branch of computer science known as artificial intelligence, machines can comprehend data, form opinions, and even interact with people. The strength of AI is the ability to learn and acquire skill in detecting patterns and relationships from vast datasets; for example, Using AI, whole medical history of any patient can be saved under single number. Furthermore, by learning more data AI is improving day by day.

Around the world, many healthcare systems face problem in fulfilling "the quadruple objective- improving population health, improving patient experience of care, improving clinicians experience, and lowering rising healthcare costs". Reforming healthcare delivery system is necessary by government as there is rising up in the number of chronic diseases as well as overall global health related expenditure. Recent COVID epidemic has made us aware of scarcity of healthcare workers and in providing care, as stated by the World Health Organisation(WHO).

By adding technology with AI, few "demand and supply problems" of healthcare system will get solved. As data in different modes like demographical, clinical etc. along with technology, Artificial Intelligence Augmented Healthcare System is changing traditional healthcare.

Cloud computing, in specific, is helping integration of use of AI systems into delivering health. When corelated to "on premises" healthcare institutions, for the processing of big data in increased speed and minimum cost, cloud computing is the best to use.

I. IMPLEMENTATION OF ARTIFICAL INTELLIGENCE IN PROSTHODONTICS

Machine learning models of AI based on analysis of previous data to simulate human intellect and behaviour. For more accurate results, training of AI models is necessary using already present data. The use of AI in prosthodontics resulted in major modifications in their role to digital diagnostics, predictive assessments. Prosthodontics incorporates nearly every aspect of current dental technology. There is increase in the number of replacing conventional impression making by digital impressions with an intraoral scanner. For making single crowns and short term FPD intraoral scanners are reliable. Recently this scanning technology is helping in complete denture fabrication and maxillofacial scanning.

Following an intraoral scan, AI was used to detect margins in fixed prosthodontics. Various fields of dentistry are currently using tools which are digital to help patients getting their beautiful smile which they always wish to have. 3D face tracking with low-cost virtual 3D data hybrids -fragmented cone beam computed tomography (CBCT), intraoral scans, and face scans are examples. Virtualizing their anatomy AI can provide treatment that helps to improve aesthetics of patients.

"According to a study, "AI applications in prosthetic dentistry and its integration with other branches of dentistry have resulted in a wide range of innovative opportunities, including the generation of occlusal morphology in crown contemplation of opposing teeth even in cases of

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wear or fracture, programmed teeth setting for dentures, and automatic framework designs for removable dental prostheses."

II. AI IN IMPLANT PROSTHODONTICS

The most effective dental implant treatment planning utilises “CBCT image and intraoral scan”. The use of Artificial Intelligence in implantology has the ability to combine both and create the prosthetics of the future.

AI based model can automatically identify the exact position of the mandibular canal for dental implant operations. Lee J et al.3 in his study conducted, panoramic and periapical radiography were implemented to segregate implants using AI- CNNs. Based on the findings of this investigation, the AI-CNN system is nearly as effective as humans in categorising implant procedure. Lerner et al. developed an AI model to reduce the risk of errors in implants such as inappropriate placement, inadequate cementation, incorrect occlusion. Takahashi et al. conducted a systematic investigation to establish an AI framework that would categorise dental arches and use CNN to aid in denture production. The training dataset was categorised using computer-based autonomous learning algorithms.

AI based deep CNN model helps in for implant type recognition using 2-dimensional radiograph and 3D CBCT images as the input data set. Clinicians attempting to restore an unidentified implant would benefit from the clinical applicability of such an AI tool. Additionally, implant dentistry specialists who lack expertise in the field can benefit from adopting software that can identify implants.

III. MAXILLOFACIAL PROSTHESES AND AI

Dental professionals can develop the most attractive prosthesis for patients with the use of AI and specific designing tools, taking into account anthropological calculations, face measurements, ethnicity, and patients demand. AI in synergy with (CNNs) mimic human neurons. Patients comprising of vision impairments used the prosthetic eye created in the United States. These AI-based gadgets assist individuals to see without surgical aid. For people who are blind or have visual impairments, there are smart reading glasses available. Being an advanced voice-activated device it can be mounted practically on any set of glasses. It is primarily intended to assist those who are blind or visually impaired. Its user can live an independent life by being able to read text from a book, smartphone screen, or any other surface quickly, recognize faces, work more efficiently.

“Bionic eye created by United States was adapted by individuals who lost their eye”. The user can read text or identify faces using a smart camera on specialized eyewear. It analyses the camera data and transforms it into sound. A wireless earpiece then transmits this sound to the blind person's ears.

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Artificial olfaction or Machine olfaction (automated simulation of the sense of smell) plays a crucial role in robotic technology because it closely resembles the human olfactory system, which is capable of identifying various odours in a variety of fields including disease diagnosis, environmental monitoring, concerns with public safety, the food business, and agricultural production.

People who have had limbs amputated could no longer be able to feel changes in temperature. Researchers created synthetic skin that altered this image. The tissue, which is composed of a thin, transparent layer of pectin and water, can detect temperature changes between 5 and 50 °C. It is done by capturing images using specialized thermal cameras or infrared cameras. AI algorithms extract relevant features from the thermal images. Features could include temperature gradients, temperature patterns, or specific regions of interest on the skin. Convolutional Neural Networks (CNNs) are commonly used for thermal image analysis.

In order to explore the potential of AI to augment, automate, and modify dentistry, we present a non-exhaustive set of applications for AI in healthcare for today and in next 5-10 years.

1. **AI Today:** AI systems are not yet capable of reasoning in the same way as doctors, who can rely on "common sense" or "clinical intuition and experience. AI, on the other hand, works more like a signal translator by translating patterns from datasets. Healthcare organisations are now starting to use AI technologies to automate time-consuming, repetitive procedures with huge volume. Additionally, there has been significant development in showing the application of AI in precision diagnoses such as planning radiotherapy.

2. **AI in next 5–10 Years:** In the medium term, we predict that there will be significant advancements in the creation of effective algorithms that can combine various types of structured and unstructured data, including imaging, electronic health data, multi-omic, behavioural, and pharmacological data, and are efficient (i.e., require less data to train). Additionally, medical practises and healthcare organisations will progress from using AI platforms as adopters to working with technology partners to construct cutting-edge AI systems for precision therapies.

   In the future, AI will be able to produce a forecast that can be used in conjunction with human diagnosis to raise the likelihood of suitable diagnostics and result in a greater rate of accurate diagnoses, according to the statement "AI helps doctors and patients in every profession." Everything is changing thanks to AI, from dentistry to space science. "Due to AI, the clinical and dental patient experience will improve". The system will learn preferences in order to enhance patient experience. Increased access to adequate oral health treatment will improve dental patient experiences, improving systemic health. The software will provide partial edentulism RPD designs. Dental implant therapy will be modernised by means of techniques and technology that have been tested in clinical settings. When there is only partial edentulism, the software will help with partial denture design.

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In conclusion, incorporation of artificial intelligence (AI) in prosthodontics offers a revolutionary advance in the fields of dental prostheses and oral healthcare. A more accurate, effective, and patient-centred approach to healthcare is being offered by AI-powered systems that are transforming diagnosis, treatment planning, and the development of personalized prosthetic solutions. AI is enabling prosthodontists to deliver higher-quality care while improving patient comfort and satisfaction. It can analyse large datasets, optimize treatment outcomes, and expedite administrative operations. As AI develops, prosthodontic procedures have the potential to change, becoming more accessible, efficient, and customised to meet the individual needs of each patient.

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TELEDENTISTRY: DENTAL CONSULTATIONS MADE MORE ACCESSIBLE

Abstract

Teledentistry, an evolving field of dentistry and telecommunications, has revolutionised oral healthcare delivery. This article delves into recent breakthroughs in teledentistry, showcasing its potential as a solution for long distance diagnosis and treatment planning. With the exchange of clinical data for remote consultations and treatment planning, teledentistry addresses enduring dental challenges while facing its own set of obstacles. The underprivileged in rural areas stand to gain quality care, ensuring oral health and well-being. Notably, the rise of real-time videoconferencing, accentuated by the COVID-19 pandemic, has highlighted the versatility of teledentistry. From bridging the rural-urban health divide to extending specialist services worldwide, teledentistry is rapidly advancing in information and communication technology, promising expert dental care even in the remotest corners of the globe.

Keywords: teledentistry; telecommunication; telemedicine; digital; real-time consultation; dentistry; COVID-19

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I. INTRODUCTION

Teledentistry, an emerging branch of dentistry that connects patients and dental professionals through telecommunication. With the rapid advancement of technology, it has the potential to drastically revolutionise the present practice of dental care. This field involves the use of telecommunication technology in dental care. The term "teledentistry" was first used in 1997, and was defined as," the practice of using videoconferencing technologies to diagnose and provide advice about treatment over a distance" by Cook. It provides a new way of providing specialist advice. With the help of this, it is now possible for patients to access professional consultation through the use of telecommunication and computer technology that are not constrained by time or space.

II. HISTORY

Telemedicine began in 1924 with the idea of a patient consulting a physician over the radio using a television screen. Teledentistry was initially started by NASA in the 1970s, followed by the US military. The initial concept for teledentistry was developed during a conference in Baltimore in 1989 that was sponsored by the Westinghouse electronics group and which was developed for informatics as a part of the blueprint. Teledentistry was first put into practice by the United States Army in 1994 to provide dental consultations to people who lived more than 100 miles apart. Since then, various institutes and organizations have used teledentistry with varying degrees of success.

III. TELEDENTISTRY

Teledentistry is the use of information technology and electronic telecommunications to facilitate oral health care in remote and rural areas through patient education and professional consultation.

It is of two types: Real-Time Consultation and Store and Forward Method.

1. **Real-Time Consultation:** This involves the use of advanced telecommunication devices, where patients and dental professionals can interact from different locations through videoconferencing.

2. **Store and forward:** This consists of the exchange of clinical information and images that are collected and stored on telecommunications equipment. The dentist obtains all the clinicalinformation, along with the radiographs, from the patient. This data is further forwarded to a professional for diagnosis and treatment planning. This thus helps provide a more precise, less time-consuming and economical treatment. In this method, the patient does not need to be present during the consultation.

IV. REQUIREMENTS

Certain technology, software, and network connections are required to practice teledentistry. A desktop or laptop with a sufficient-sized hard drive and RAM and a quick processor is required. The consulting dentist can be provided with images of great clinical value using digital, intra-oral, or video cameras, and intra and extra-oral x-ray units are necessary. For consultations through videoconferencing, speaker, microphone, and webcam are required.

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V. APPLICATIONS OF TELEDENTISTRY

1. **Oral Medicine and Radiology:** With the advent of teledentistry, access to diagnosis and management of various dental problems has become easier. Early diagnosis of oral lesions such as pre-malignant lesions and vesiculobullous lesions can be made by analysing the images sent by the patient to the consulting dentist. This enables the dentist to provide appropriate instructions to the patients for the management of the condition and to prescribe medication through teleprescription. Radiographs such as intra-oral radiographs, Digital Orthopantomogram (OPG) and Cone Beam Computed Tomography reports can be sent to the dentist for early diagnosis and treatment planning of cysts, tumours, and fractures. Tobacco cessation counselling can also be provided through telecommunication. Moreover, teledentistry aids in the diagnosis of oral conditions even in remote areas.

2. **Oral and Maxillofacial Surgery:** In this branch, teledentistry plays a major role in the consultation of patients presenting with pain due to 3rd molar impaction, which is the most common complaint oral surgeons come across. The consulting dentist can forward the images and radiographs of the patient to the specialist for consultation in case of complications or a second opinion. This can also be helpful in providing economical pre-operative and post-operative evaluation of the patients in cases where transport is not feasible. Some studies reported that patients with trauma generally accepted teledentistry well, while patients with temporomandibular joint disorders showed low acceptance. Furthermore, as reported by Saad Ahmed and Omar, teledentistry is advantageous for oral surgery, both for performing dental treatments and for following up on should have been patients' ostoperative status.

3. **Orthodontics:** With technological advancement, teledentistry has become popular in orthodontics to monitor and consult patients without having to visit the dentist. It can be helpful in early orthodontic consultations for the evaluation of treatment options and diagnostic plans. Minor orthodontic emergencies such as displacement of the rubber ligature, broken and exposed wire, or bracket breakage causing pain and irritation to the cheek, can be advised to be managed at home. According to Berndt *et al*. teledentistry aided in the provision of interceptive orthodontics by general dentists under the guidance of orthodontists. In case of low-income households that do not have proper access to dental care, teledentistry can help reduce the chances and severity of malocclusion. Despite the fact that teledentistry has not totally eliminated the need for in-person clinical care, most dentists and patients think that online consultations or teledentistry are more convenient and cost-effective for orthodontic treatment.

4. **Endodontics:** Teledentistry enables access to endodontic care even for the underprivileged sections of society. Patients presenting with the common complaint of pain and swelling can describe their symptoms on-call to the dentist. Further, the dentist can prescribe medications like analgesics and antibiotics to reduce the symptoms. According to Brullmann D *et al.* remote dentists can identify root canal orifices using images of endodontically accessed teeth. Zivkovic D *et al.* demonstrated that the diagnosis of the pulp and periapical lesions of the anterior teeth can be successfully done through teledentistry. It offers the additional benefit of lowering the expense of distant visits and increasing the availability of emergency treatment.

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5. **Prosthodontics:** In prosthodontics, dislodged prosthesis and broken dentures are the most frequently reported problems. These problems can be managed at home by the patients with the guidance provided by the dentists. In cases of broken dentures the dentist can coordinate with a laboratory technician for the collection and repair of the denture. On-call consultations can also include instructions on denture care and hygiene.

6. **Pediatric and Preventive dentistry:** Oral health has a significant impact on a child‘s overall well-being. Pediatric dentists can encourage their patients to practice improved dental hygiene habits like proper brushing techniques and use of fluoridated toothpastes with the help of teledentistry. They can even provide counselling over the phone to start treatment regimens. As dental caries is more common in children, the extra dental visits can be reduced through teledentistry. Parents can describe their child‘s symptoms and also send pictures to the dentist. The dentist can then assess the severity of the situation and provide an appropriate treatment plan for the child. In situations of dental trauma at home, parents can be instructed to apply cold packs to the site of injury, and in cases of avulsed teeth, the tooth can be stored in milk. The patient can be given an appointment to visit the clinic for re-implantation of the avulsed tooth.

7. **Periodontics:** In the branch of periodontics, the store and forward method of teledentistry can be used to formulate treatment plans for patients. Images and radiographs of patients with gingival and periodontal problems can be sent to the specialist for further consultation. The dentists can provide instructions on-call regarding proper brushing techniques and the use of various oral hygiene aids.

VI. ADVANTAGES OF TELEDENTISTRY DURING THE COVID-19 LOCKDOWN

During the COVID-19 lockdown, many dental offices and institutions temporarily suspended elective treatment in order to limit the spread of the infection. During this time period, there was a rapid shift towards teledentistry, as this helped provide a safe environment by decreasing the chances of infections. Teledentistry can be used as an effective medium for expert consultations, monitoring patients, and providing treatment plans, decreasing the number of patient visits. Minor emergencies can also be managed by the dentist prescribing analgesics and antibiotics, which can help reduce the symptoms until the lockdown is lifted.

VII. INFROMED CONSENT IN TELEDENTISTRY

Informed consent is an important aspect of the doctor-patient relationship in the medical field. In teledentistry, informed consent should include all the aspects of the standard and conventional consent forms. The consent should also provide information to the patient regarding the risk of treatment and diagnostic errors that can occur due to technical failure. The practitioners of teledentistry should take all the necessary precautions to avoid patient privacy being compromised by unauthorized persons. Despite the practitioner's best efforts to ensure security, patients should be informed that their information is conveyed electronically and that there is a potential that it may be intercepted. The names of the referring and consulting doctors should be listed on the consent form, and the consulting doctor should receive a copy of the consent form before beginning any type of patient engagement in order to prevent malpractice during the course of treatment. The copyright and medico-legal aspects must also be considered while practicing teledentistry. The reliability, security,

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efficacy, or efficiency of the information transferred cannot yet be ensured. Along with financial, payment, and tax obstacles, electronic commerce raises privacy and security issues. Legislative or judicial departments of numerous governments have yet to definitively decide on several legal issues, including licensure, jurisdiction, and malpractice.

VIII. SCOPE OF TELEDENTISTRY

Teledentistry can help improve the access and provision of oral health care while being cost-effective. It has the ability to close the gap between rural and urban communities in terms of oral healthcare. With the advancement of technology and telecommunications, teledentistry can now help provide specialized healthcare to people in remote parts of the world. According to Lienert N *et al.* at a Swiss telemedical center, telemedical services were beneficial for cases involving dental trauma and offered crucial support in cases where a specialist was not present.

Teledentistry aids in diagnosis and management by sharing clinical and radiological images with the specialist consultant. It also plays an important role in providing second opinions for complicated cases. Moreover, it helps in getting second opinions because pre-authorization and other assurance criteria may be quickly accomplished online utilizing actual dental issues as opposed to tooth charts and textual descriptions. Additionally, teledentistry presents a chance to enhance conventional teaching strategies in dental education and create new options for dental professionals and students.

IX. DISCUSSION

Teledentistry is an emerging aspect of patient treatment that is quickly gaining acceptance and value. The exchange of information through teledentistry will result in better patient care, and the capacity to consult with colleagues more effectively will result in better knowledge of the treatment objectives and better treatment outcomes.

There are still potential shortcomings of teledentistry, including the necessity for adequate training, a quick response, misunderstanding of messages, concerns regarding privacy, and the possibility of overlooking the messages. Teledentistry practitioners must be aware of the technological, legal, and ethical challenges that may arise. The dentists must be well-versed with the technology and should know its effect on both patients and the dentist. It is possible to incorporate teledentistry into professional dental education, which will aid in developing teledental skills. Additionally, teledentistry education course instructors should be knowledgeable about computers in addition to having teaching expertise.

Concerns about the cost of the telecommunications equipment have also been raised. Following a 12-month experiment of teledentistry, Scuffham PA and Steed M arrived at the conclusion that with the increased familiarity and use of equipment would improve teledentistry's cost-effectiveness. The availability of intraoral cameras, digital cameras, and computers with internet connectivity in almost all dental offices has now made teledentistry easier to use. The price of teledental consultations has decreased as technology has advanced due to changes in the size, features, and cost of various technical components.

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X. CONCLUSION

Teledentistry carries immense promise and potential in the field of dentistry. The applications of teledentistry transcend the immediate crisis, reaching into realms such as long-distance clinical training, ongoing education, and proactive screenings. This transformative approach not only brings extended and cost-effective quality care to remote patient groups but also addresses the scarcity of specialized dental expertise in underserved rural areas. By granting primary care professionals' swift access to efficient consultations, it opens new horizons for postgraduate learning and continuous improvement. It promises a future where oral healthcare transcends physical constraints, offering inclusive and accessible solutions to patients everywhere.

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Chapter - 1
A Review on Neurophysiology of Orofacial Pain and Potential Benefits of Herbal Plants for Overcoming Dental Pain

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Chapter - 1

A Review on Neurophysiology of Orofacial Pain and Potential Benefits of Herbal Plants for Overcoming Dental Pain

Dr. Angela Priyanka Gogoi and Priyangshu Sagar Dehingia

Abstract

Dental pain, a complex experience with sensory, emotional, and motivational dimensions, drives individuals to seek care for pulpal and periapical pains. Orofacial pain is categorized by duration (acute, chronic) and causes (nociceptive, inflammatory, neuropathic). Dentin hypersensitivity involves fluid-induced nociceptor activation. Orofacial pain pathways include trigeminal axons, sympathetic fibers, and discussions on parasympathetic roles. Sensory fibers, especially A-delta and C fibers in dental pulp, contribute to nociception. Pulp nociception involves neural and vascular structures.

Common herbs like ginger in India offer antibacterial, anti-inflammatory, and antioxidant benefits for oral health. Ginger addresses conditions like oral candidiasis, aphthous stomatitis, gingival issues, dentin hypersensitivity, and halitosis. Clove oil, rich in eugenol, provides anti-inflammatory benefits via TRPV1 receptors. Belladonna, a muscarinic antagonist, proves clinically useful despite potential toxicity. Homeopathic remedies like belladonna effectively treat dental issues including abscesses, bruxism, cellulitis, dry socket, trismus, and pericoronitis. Understanding dental pain complexities and exploring natural remedies like ginger, clove oil, and belladonna offer potential alternative treatments for oral health.

Keywords: Herbal plants, oral application, antibacterial activity, anti-inflammatory

Introduction

Pain, particularly in the context of dental discomfort, is a complex phenomenon with various dimensions, creating a nuanced and multifaceted experience. It involves not only sensory reactions but also emotional, conceptual, and motivational aspects. Dental care is often sought due to pulpal and periapical pains. Historically, the dental pulp has been perceived as a

densely innervated and well-vascularized tissue, evoking a solitary sensory reaction-pain-when stimulated. The neural aspect of pulp tissue comprises sensory axons from the trigeminal nerve, with sympathetic efferent fibers overseeing blood flow regulation. The function of parasympathetic fibers remains a subject of continuous debate without reaching a unanimous agreement.

Categorization of orofacial pain and possible mechanisms

The categorization of orofacial pain based on durations includes acute pain and chronic pain. In terms of causes, it can be classified into nociceptive, inflammatory, and neuropathic pain.

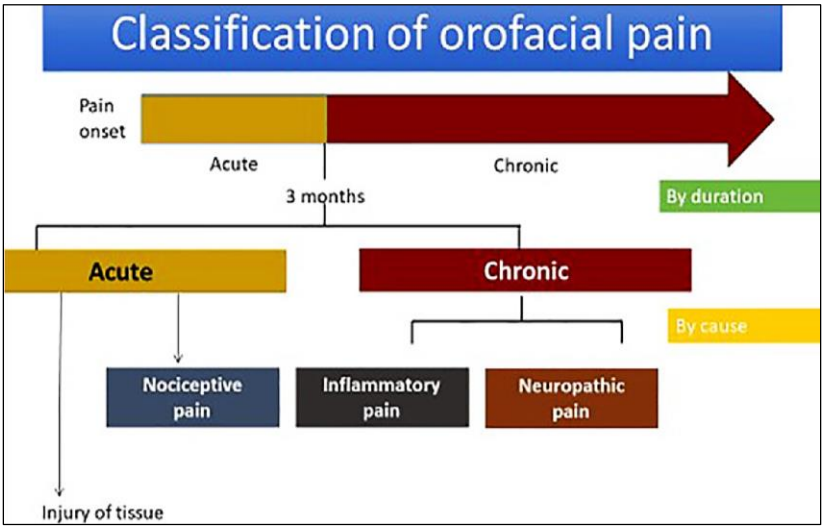


Fig 1

Acute orofacial pain denotes the abrupt initiation of pain linked to physical sensations, typically of short duration and temporary, arising from tissue injury. On the other hand, chronic orofacial pain persists beyond three months, surpassing the typical healing period. Orofacial pain can be classified into three distinct categories based on causative factors: nociceptive, inflammatory, and neuropathic pain. Nociceptive pain emerges from the direct stimulation of nociceptive sensory neurons by noxious stimuli such as heat, cold, intense mechanical force, and chemical irritants. These nociceptors then transmit signals to the central nervous system, triggering a pain response, such as the withdrawal reflex. Consequently, nociceptive pain mechanisms play a crucial role in physiological sensation. In contrast, inflammatory pain arises from tissue damage, where inflammatory mediators released after an injury

activate pain perception in the affected tissues. Neuropathic pain is caused by defects in the peripheral or central nervous system [1].

Dentin hypersensitivity: Dentin hypersensitivity is characterized by pain that arises from exposed dentin, typically triggered by chemical, thermal, tactile, or osmotic stimuli, and cannot be attributed to any other dental defect or pathology. The hydrodynamic theory is widely accepted as the most plausible explanation. According to this theory, the sensitivity of dentin is associated with fluid flow induced by stimuli in the dentinal tubules, leading to the activation of nociceptors in the border region of pulp and dentin. The proposal suggests that myelinated A-β and some A-δ fibers within the tooth respond to stimuli, causing displacement of fluid in dentinal tubules and resulting in the distinct short and sharp pain associated with dentin hypersensitivity. The specific fluid movement can be quantified by assessing the hydraulic conductance of dentin. Therefore, dentin with high conductance exhibits low resistance, while dentin with low conductance demonstrates higher resistance. Human studies have indicated that the patency of dentinal tubules is a crucial characteristic of sensitive dentin, with a significant positive correlation between tubule density and pain responses from exposed cervical dentin surfaces.

Distinguishing reversible pulpitis from dentine hypersensitivity may not be immediately apparent; however, reversible pulpitis often presents with obvious signs such as a carious cavity or crack that indicates pulpitis. In contrast to pulpitis, the pain associated with dentin hypersensitivity is brief and sharp.

Physiology of the orofacial pain pathway

The orofacial region comprises the oral cavity (including teeth, gingiva, and oral mucosa), face, jaw bone, and temporomandibular joint. The physiology of orofacial pain pathways encompasses primary afferent neurons, pathological alterations in the trigeminal ganglion, nociceptive neurons in the brainstem, and higher brain functions that control orofacial nociception. Sensory innervation in this area is supplied by the trigeminal nerve or cranial nerve V (CN V). In the peripheral nociceptors of the orofacial region, when exposed to repetitive noxious stimuli or excessive, uncontrollable inflammation, the first-order neurons in the trigeminal nerve undergo an increase in pain signals, which are then transmitted to the trigeminal ganglia. It is noteworthy that the trigeminal ganglia shares similarities with the dorsal root ganglia [1].

After that, the pain signals are sent to the second order neurons in the trigeminal nucleus caudalis located in the brainstem. The trigeminal nucleus

caudalis is similar to the dorsal horn of the spinal cord and contains second order neurons. Then the signals will be further projected to the third order neurons in the thalamus via the ventral trigeminothalamic tract.

Sensory nerve fibers within the dental pulp

The sensory nerve fibers found in the dental pulp serve as afferent endings of the trigeminal cranial nerve. These fibers travel through the apical foramen to reach the root canal, forming lumps as they proceed to the root pulp. Often associated with blood vessels in a collagen sheath, these lumps collectively form the neurovascular bundle. While there are minimal bifurcations in the root canal, upon reaching the coronal pulp, the nerve endings begin to divide, sending branches into the surrounding dentin. As these fibers reach the subodontoblastic region, they form a complex network referred to as the plexus of Raschkow. Following this, the myelinated fibers lose their myelin sheath and appear as free nerve endings. Studies carried out by Gunji from 1982 to 1988 demonstrated that a substantial number of nerve fibers conclude within the extracellular space of the rich cell zone or in the odontoblast layer. Others extend into the predentin or the dentinal tubules, penetrating up to 150 µ. In the pulp horns region, approximately 25% of tubules contain these intratubular fibers, whereas in other parts of the dentin crowns, the presence decreases to about 15%. In the root, only around 10% of the tubules contain nerve fibers, which tend to be smaller and do not extend beyond the predentin [2]. These responsive fibers serve as nociceptors and can be distinguished into two categories based on their diameter, conduction velocity, and role: A∂ (myelinated) and C (unmyelinated). Noteworthy is the fact that approximately 7% of myelinated fibers entering the pulp of human premolars fall into the Aβ category [2].

A Fibers

Myelinated axons, known for their rapid conduction speed and low stimulation threshold, are located near the junction of the pulp and dentin. These axons directly convey pain signals to the thalamus, leading to a distinct and piercing pain sensation that is readily localized. These fibers, due to their distinctive characteristics, are the first to react and transmit pain impulses, even in the absence of irreversible tissue damage. A-delta fibers display key clinical characteristics, reacting to hydrodynamic stimuli such as drilling, sweet foods, cold air, and hypertonic solutions, resulting in swift fluid movement within the tubules [3]. This stimulation activates mechanosensitive nerve endings, causing a brief, sharp initial pain. While slow outward capillary fluid movement typically does not trigger pain, more intense and rapid fluid flow, as in desiccating or drying the dentin, is likely to activate pulpal

nociceptors [4]. Therefore, any factor increasing fluid movement by opening dentinal tubules is expected to heighten dentine sensitivity, as observed in clinical scenarios like cutting a cavity or exposing open root dentin through scaling or toothbrush abrasion.

Within the A-delta fiber population, arousal thresholds vary. Low-threshold fibers respond to stimuli like cooling and vibration, which are seldom associated with nociception, possibly being involved in reflex or perception-related functions. Higher-threshold A fibers respond to stronger stimuli, such as mechanical instrumentation, and function as nociceptors [5]. A-beta fibers are considered functionally similar to A-delta fibers, although they respond differently to vibration, stimulated at a lower electrical threshold [6]. The receptive field of these fibers, or the site responding to stimulation, is located at the pulp-dentine border or in close proximity to the odontoblast cell body.

C Fibers

C fibers are unmyelinated and possess a low conduction velocity, smaller diameter, and higher excitation threshold. Situated deeper than myelinated fibers, they are primarily activated by heat, resulting in slow, diffuse, and persistent pain. An increase in pain stimulus intensity recruits sensory C fibers, transforming the pain into a burning sensation. The reaction of C fibers indicates irreversible pulp damage. The presence of A-delta fibers in nerve bundles within the central region of the pulp elucidates the phenomenon of referred pain from a particular tooth, as nerve fibers extend to multiple teeth with diverse pulps [7].

Unlike A fibers, C fibers maintain functional integrity when the tissue becomes hypoxic due to lower oxygen consumption. Consequently, in cases of injury leading to pulp microcirculation interruption, C fibers continue to function longer compared to A fibers, which are inactivated or infarcted. Clinically, this pain is described as dull, vaguely located, and aching, intensifying several seconds after a hot drink. This characteristic explains situations where a tooth negatively responds to a cold CO_2 stick test (due to degenerated A-delta fibers) but is painful to mechanical instrumentation at the start of endodontic therapy. It is recommended to administer local anesthesia when a patient experiences pain in reaction to hot beverages (indicating the presence of active C-fibers) but shows a negative response to cold or electric sensitivity tests. The types of fibers can be related to various clinical pulp testing methods:- Thermal pulp testing relies on dentinal fluid movement, while electric pulp testing depends on ionic movement [8].

- A-delta fibers, stimulated in electric pulp testing due to their distribution, larger diameter, conduction speed, and myelin sheath, respond more strongly [8].
- C fibers do not respond well to electric pulp testing, requiring a stronger electric current due to their high threshold.
- Hydrodynamic effects of cold (fluid contraction) produce a stronger response in A-delta fibers compared to heat (fluid expansion) [3].
- Repeated cold applications reduce fluid displacement, causing a less painful pulp response, leading to a refractory cold test.

A-delta fibers are more affected by reduced pulpal blood flow compared to C fibers, unable to function in anoxic conditions. Unregulated heat tests may harm the pulp and release mediators that influence C fibers. The presence of a positive percussion test suggests that inflammation has transitioned from the pulp to the periodontium, provoking a localized response [2]. Pulp vitality electrical tests in immature teeth may yield unreliable results due to a large number of C fibers in early tooth development, increasing A fibers with age. All reversible functional changes in nociceptors are resolved upon removing the cause, as seen in dentin hypersensitivity treatment, which directly affects A fibers (hydrodynamic cessation) and resolves neural changes in the pulp, alleviating pain [9].

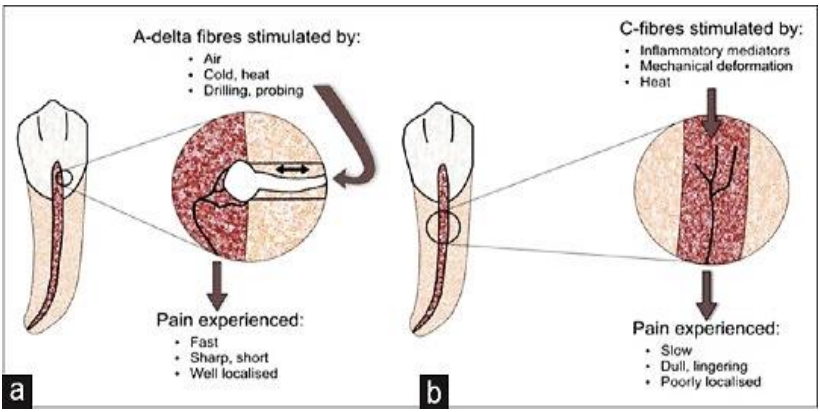


Fig 2

Molecular foundations of pulp nociception

The origin of dental pain lies in the activation of nerve fibers within the dental pulp. Nociception involves the participation of both neural and vascular components. As a sophisticated response, this protective mechanism encompasses various systems that collectively contribute to its initiation and control.

Potential benefits of commonly found herbs in India

1. Ginger in maintenance of oral health

Ginger is a rich source of active compounds, including phenolic compounds like Gingerol and Shogoal, volatile oils such as zingiberene and zingiberol, sesquiterpene hydrocarbons, oleoresins, and Diarylheptanoids like Gingerenones A and B. It can be utilized on its own or in combination with other natural products for desired therapeutic effects. In terms of oral health, ginger has demonstrated an inhibitory effect on microorganisms responsible for tooth decay. It acts as a dental anaesthetic and can contribute to dentine remineralization. The antiplaque and oral deodorizing properties of ginger help maintain clean and fresh teeth, preventing tartar and caries [10].

Sesquiterpene hydrocarbons are frequently identified terpene components, and the combination of terpenes and oleoresin forms ginger oil. Ginger comprises approximately 1% to 3% volatile oils and non-volatile pungent components known as oleoresin. Additionally, ginger is rich in minerals, vitamins, carbohydrates, protein, fats, soluble and insoluble fibers, and phytochemicals. Gingerols exhibit various pharmacological activities, including antioxidant, anti-inflammatory, and antimicrobial effects [10].

1.2 Properties of ginger

Gingerols, obtained from ethanol and n-hexane extracts of ginger through alkylation, have been identified to exhibit antibacterial, anti-tumorigenic, antioxidant, and anti-inflammatory properties (Figure 2). Notably, their antibacterial efficacy against Staphylococcus aureus and Streptococcus pyogenes surpasses that of commonly available antibiotics.

1.2.1 Anti-inflammatory property

The inhibitory effects of ginger on prostaglandin synthesis, marking its anti-inflammatory activity, were first reported in the early 1980s [11]. The pharmacological effects of Gingerdiones and Shogaols in ginger are comparable to non-steroidal anti-inflammatory medications (NSAIDs) [10, 11]. Owing to its lower negative effects compared to traditional NSAIDs, ginger is being explored as a potential new family of anti-inflammatory solutions. Ginger prevents the breakdown of arachidonic acid via the cyclooxygenase and lipoxygenase pathways, inhibiting the stimulation of genes involved in the inflammatory response and those encoding cytokines, chemokines, and the inducible COX-2 enzyme (cyclooxygenase-2). The volatile oils in ginger influence both cell-mediated immune responses and nonspecific T lymphocyte proliferation, modifying lymphocyte and cellular immune

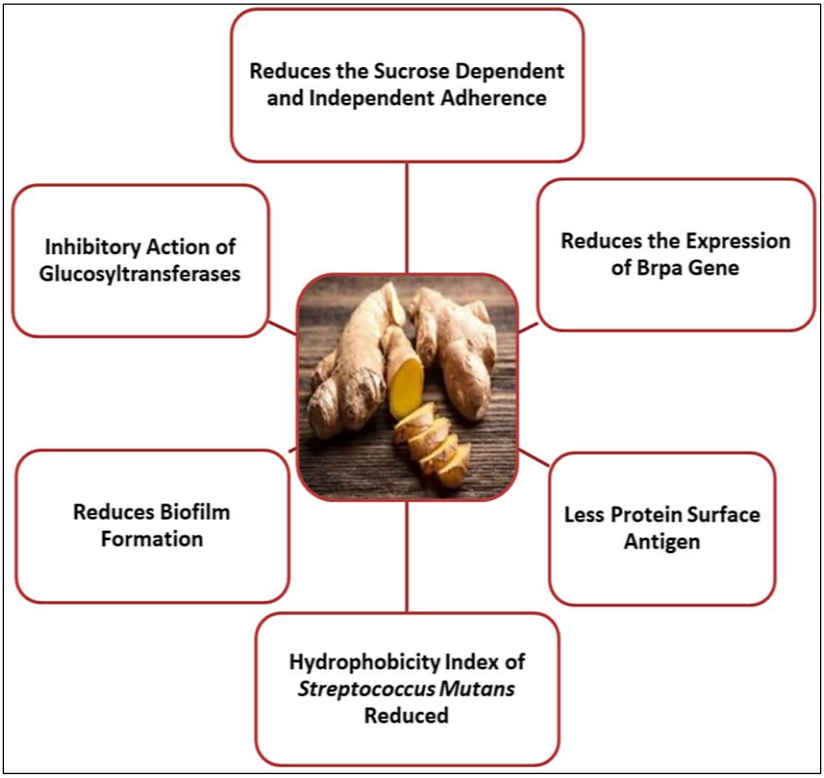
responses. This imparts positive benefits in various clinical situations, including chronic inflammation and autoimmune disorders [10].

1.2.2 Antioxidant property

Ginger, rich in antioxidants like ginger essential oil and oleoresins, exhibits concentration-dependent scavenging of 1,1 Diphenyl, 2 picrylhydrazyI (DPPH) free radicals. The antioxidant activity of ginger extract plays a protective role against radiation toxicity, lethality, and various toxic chemicals such as carbon tetrachloride and cisplatin. It also serves as an anti-ulcer drug. In relation to Fe3+, ginger displays strong chelate-forming ability, preventing the formation of hydroxyl radicals known as lipid peroxidation inducers. The main antioxidant components of ginger include polyphenols, tannins, and flavonoids [10].

1.2.3 Antibacterial property

Gas chromatography-mass spectrometry research reveals that ginger essential oil and oleoresins contain significant levels of phenolic chemicals, contributing to antibacterial activity. The antibacterial activity against oral pathogens, particularly Streptococcus mutans, involves various mechanisms. Ginger extract affects the sucrose-dependent (SD) and sucrose-independent (SI) adherence of Streptococcus mutans by blocking the enzyme glucosyltransferases (GTPase), hindering the conversion of sucrose into sticky glucans for tooth surface adherence. The presence of ginger extract significantly reduces the index of hydrophobicity, preventing bacterial adherence to surfaces. Biofilm formation is hindered due to reduced hydrophobic interactions, and the activity of enzymes engaged in physiological processes is impaired, potentially leading to the destruction of Streptococcus mutans. Ginger extract also downregulates the expression of pathogenic genes, including relA, brpA, gtfC, and comDE, involved in oxidative stress, acid tolerance, biofilm production, and quorum-sensing. Gingerol and shogaol from ginger rhizomes exhibit antibacterial properties against anaerobic bacteria causing periodontal disease in the oral cavity [14].



Source: Kermode, I. (2022) Ginger and its health benefits. New York: Nova Science Publishers, Inc.

1.3 Oral applications of ginger

1.3.1 Oral candidiasis

Oral candidiasis, a prevalent fungal infection affecting the oral cavity, occurs when Candida albicans accumulates on the mucosal lining, resulting in white lesions. Traditional topical and systemic antifungal treatments often face drug resistance, diminishing their clinical efficacy. To address this challenge, researchers investigated ginger's potential as an antifungal agent against Candida albicans, a primary cause of oral candidiasis. In a study by Khalaf *et al*., alcoholic extracts from ginger were found to be effective against Candida albicans, suggesting its potential for treating oral candidiasis. The study identified CMUAC 130, a substance in ginger roots, which demonstrated inhibitory effects on Candida albicans' biofilm formation. To assess its antifungal properties, ginger was compared with other medications such as fluconazole, amphotericin B, and nystatin in preventing Candida albicans colonization. Results indicated inhibition zones of up to 18, 21, and

25 mm at concentrations of 50, 10, and 10 mg/mL of the crude extract, respectively. Consequently, ginger shows promise as a topical antifungal agent when incorporated into oral medications.

1.3.2 Apthous stomatitis

Aphthous Stomatitis, a common condition affecting the oral cavity, manifests as a benign disorder characterized by numerous small, circular oral ulcers. Ginger, known for its antipyretic properties, has demonstrated suitability as an analgesic in addressing this condition. In a study, it was shown that the application of dried ginger rhizome (DGR) on aphthous ulcers resulted in analgesic effects. DGR was identified as a potent agonist of TRPV1 channels (Transient Receptor Potential Cation Channel Subfamily V Member 1), inducing a warm sensation by sensitizing sensory endings in inflamed ulcer tissues before triggering pain sensitivity. This mechanism is believed to contribute to the relief of ulcer-related pain. The study proposes that DGR can be used in the treatment of aphthous stomatitis to provide analgesia, support wound healing, and exert immune-suppressive effects. This proposition is supported by a study conducted by Parya *et al*., where a mucoadhesive system containing ginger was evaluated for its effectiveness in treating aphthous stomatitis, revealing a significant reduction in discomfort and a substantial decrease in the inflammatory area.

1.3.3 Gingival and Periodontal conditions

Gingivitis occurs when subgingival bacteria disrupt the gingival tissue, leading to inflammation of the sulcular epithelium's barrier function. Pathological changes in gingivitis are associated with oral sulcus and bacterial pathogens attaching to the tooth. Notably, Prevotella intermedia, *Porphyromonas gingivalis*, and *Porphyromonas endodontalis* are known to exacerbate gingivitis and periodontitis, which is further supported by the recognized antimicrobial properties of ginger. Consequently, ginger can be utilized in mouthwashes or as a powder to address gingival and periodontal issues. Its antimicrobial action involves the suppression of COX-1 and COX-2 enzymes, leading to reduced prostaglandin biosynthesis and a decrease in the proliferation of anaerobic Gramnegative periodontal bacteria, including *Prevotella intermedia*, *Porphyromonas endodontalis*, and *Porphyromonas gingivalis*. This assertion is backed by a randomized controlled study conducted by Anshula *et al*., which observed a notable reduction in both plaque score and gingival index among children who utilized ginger mouthwashes. Similar positive outcomes were noted in patients with gingivitis when comparing the effectiveness of ginger mouthwashes to chlorhexidine mouthwashes.

1.3.4 Anti-caries agent

Utilizing ginger independently, especially in toothpaste, offers various advantages, including anti-caries properties, enhanced dentine hardness, remineralization potential, reduced sensitivity, and acting as a polishing agent after oral prophylaxis. Dental caries is a prevalent oral ailment closely linked to the progression of Streptococcus sanguinis and Streptococcus mutans. Investigations into the antibacterial effects of ginger extracts on these bacteria reveal that methanol and ethyl acetate extracts exhibit antimicrobial activity against Streptococcus mutans and Streptococcus sanguinis. Ginger's anti-biofilm, anti-adhesion, and bactericidal capabilities contribute to diminishing dental caries occurrence. This corresponds with a study by Jain *et al*., emphasizing the highest antibacterial activity of an organic solvent-based ginger extract against Streptococcus mutans. Research by Celic *et al*. underscores ginger's potential for tooth remineralization, presenting promising results. Additionally, another study highlights the preventive anti-cariogenic potential of zerumbone, an active ginger component, with a minimum inhibitory concentration of 250 g/mL and a minimum bactericidal concentration of 500 g/mL against Streptococcus mutans. However, an examination of ginger's impact on dentin stiffness and hardness, comparing it with sodium hypochlorite and Ethylenediaminetetraacetic acid treatments, indicates a reduction in dentin micro-hardness with ginger (oil) compared to the other substances.

1.3.5 Halitosis management

Halitosis or Band Breath is a foul odour originating from oral cavity. Since ginger has antimicrobial property, it can be used in mouthwash to reduce halitosis. This can be achieved due to the presence of Phytochemicals.

2. Benefits of clove oil (primarily eugenol)

The primary therapeutic effects of clove oil are attributed to its major component, eugenol, with acetyl eugenol and eugenol playing pivotal roles in providing anti-inflammatory and analgesic benefits. The antimicrobial properties of clove oil predominantly arise from eugenol, oleic acid, and lipids present in its composition. Bley's study underscores eugenol's analgesic activity, emphasizing its anti-nociceptive potential through TRPV1 receptors. Continued activation of these receptors not only amplifies the permeability of calcium ions but also heightens their release from intracellular organelles. Prolonged exposure to eugenol and TRPV1 may lead to impaired local nociceptor function due to elevated intracellular calcium levels, affecting cytoskeletal components like microtubules. Consequently, variations in

concentration can result in diverse effects, ranging from analgesic to anaesthetic.

3. Use of belladonna in oral pain

In the realm of oral pain, belladonna, derived from the deadly nightshade plant, exerts clinical and toxic effects through an anticholinergic mechanism that affects both the central and peripheral nervous systems. Atropa belladonna contains alkaloids such as hyoscyamine, scopolamine, and primarily, atropine, serving as naturally occurring muscarinic antagonists. Atropine, a non-selective muscarinic antagonist, is commonly used as an adjunct for anaesthesia. The anticholinergic actions of belladonna encompass inhibiting secretions, resulting in dry mouth, inducing tachycardia, causing pupillary dilation and paralysis of accommodation, relaxing smooth muscles in various organs, and inhibiting gastric acid secretion. Belladonna's active components act as competitive antagonists at muscarinic receptors, preventing acetylcholine binding in the central nervous system and parasympathetic postganglionic muscarinic receptors.

Despite its potential toxicity, belladonna can be clinically valuable for anamneses in clinical examinations and detailed imaging, especially in cases resembling acute cerebrovascular events. Poisoning from Atropa belladonna should be considered in geriatric patients exhibiting symptoms such as hallucination, meaningless hand gestures, and altered consciousness.

Several homeopathic remedies, including belladonna, have proven effective in treating various dental conditions. Belladonna is particularly recommended for early dental abscesses accompanied by redness and throbbing pain, as well as for addressing issues like bruxism (teeth grinding), cellulitis, dry socket, trismus, and pericoronitis. It has demonstrated efficacy in managing various gum diseases.

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Chapter - 2
Odontogenic Benign Tumors Common in Jaws and Treatment Methods

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Chapter - 2

Odontogenic Benign Tumors Common in Jaws and Treatment Methods

Muhammet Yasin Pektaş

Abstract

The term "tumor" encompassed any swelling or mass within the body. These growths are classified into two categories: benign tumors, which typically exhibit slow growth, and malignant tumors, characterized by rapid proliferation[1]. Benign tumors, due to their gradual expansion, may displace tooth roots and cause root resorption, whereas malignant tumors spread swiftly, resulting in bone destruction without altering tooth positions but still causing root resorption. Enclosed within a capsule, benign tumors tend to maintain a rounded or oval shape as they grow, exerting pressure on surrounding tissues. In contrast, malignant tumors exhibit invasive behavior, extending beyond their initial site and infiltrating neighboring tissues. This invasive tendency has led to cancer being likened to a crab, as it penetrates tissues like claws digging into flesh[2]. The term "cancer" itself originates from the Latin word for crab, reflecting this aggressive nature. In this chapter, odontogenic benign tumors and their treatment methods, which are common in jaws are explained.

Keywords: Odontogenic benign tumors, treatment methods, jaws.

Introduction

Odontogenic tumors comprise a diverse group of lesions characterized by various histopathological types and clinical behaviors. While predominantly benign, malignant forms also exist within this category. Certain benign tumors may exhibit persistent local recurrences due to their lack of well-defined boundaries and infiltrative nature. Some are considered true neoplasms and rarely display malignant tendencies. Additionally, there are malformations described as "Hamartoma", which resemble tumors but are not true neoplasms [1, 2]. The classification of odontogenic tumors, as outlined by the World Health Organization in 1992, serves as the basis for understanding this subject. Some odontogenic tumors consist solely of odontogenic epithelium without

involvement of odontogenic ectomesenchyme, while others comprise both odontogenic ectomesenchyme and epithelium, referred to as Mixt Odontogenic Tumors. Dental hard tissues may or may not be present in these lesions. A third group of odontogenic tumors consists exclusively of odontogenic ectomesenchyme, with odontogenic epithelium playing a secondary role in their pathogenesis [1, 3].

1. Tumors originating from odontogenic epithelium

1.1 Ameloblastoma

Ameloblastoma, a slow-growing tumor originating from odontogenic epithelium, displays local invasiveness primarily arising from undifferentiated enamel tissue. It ranks second in frequency among odontogenic tumors, following odontoma, with a prevalence of 18% [4]. Although documented literature suggests an equal distribution across genders, it tends to be more prevalent in males. Exhibiting infiltrative local growth and potential destruction of neighboring structures, ameloblastoma stands as the sole odontogenic tumor with life-threatening implications, despite its benign categorization, owing to its capacity for metastasis. Ameloblastoma may progress into malignant ameloblastoma or ameloblastic sarcoma. In 2017, the World Health Organization (WHO) categorized ameloblastoma among benign epithelial odontogenic tumors, delineating four subtypes: conventional solid (multicystic intraosseous) ameloblastoma, unicystic ameloblastoma, non-osseous peripheral ameloblastoma, and metastasizing ameloblastoma [5]. The distribution among these subtypes is as follows: conventional solid ameloblastoma accounts for 91%, unicystic ameloblastoma for 6%, non-osseous peripheral ameloblastoma for 2%, and metastasizing ameloblastoma for 1% of cases [4, 6].

1.1.1 Conventional solid ameloblastoma

Conventional solid ameloblastoma, representing 86% of all cases, presents as multilocular radiolucent lesions, typically affecting individuals of both genders in their third and fourth decades of life. Typically asymptomatic and slow-growing, this tumor often eludes detection until observed in radiographic screenings at a small size, commonly presenting as painless swelling and jaw expansion. Despite its potential for significant size, occurrences of pain and paresthesia are infrequent. Predominantly found in the mandible (85% of cases) and less frequently in the maxilla (15%), it manifests with buccal and lingual cortical expansion, root resorption of adjacent teeth, and occasionally impacted teeth. Radiographically, it may resemble a cystic lesion, though it shares features with various odontogenic

and nonodontogenic lesions. Treatment options encompass curettage, simple enucleation and marginal block resection, with the latter becoming increasingly favored due to the tumor's tendency to infiltrate trabecular bone [5]. Despite treatment, recurrence rates range from 55% to 90%, emphasizing the importance of resection beyond radiological borders. While typically non-lethal, its infiltrative nature can pose risks if it advances into vital tissues, albeit malignant behavior is rare [4, 6].

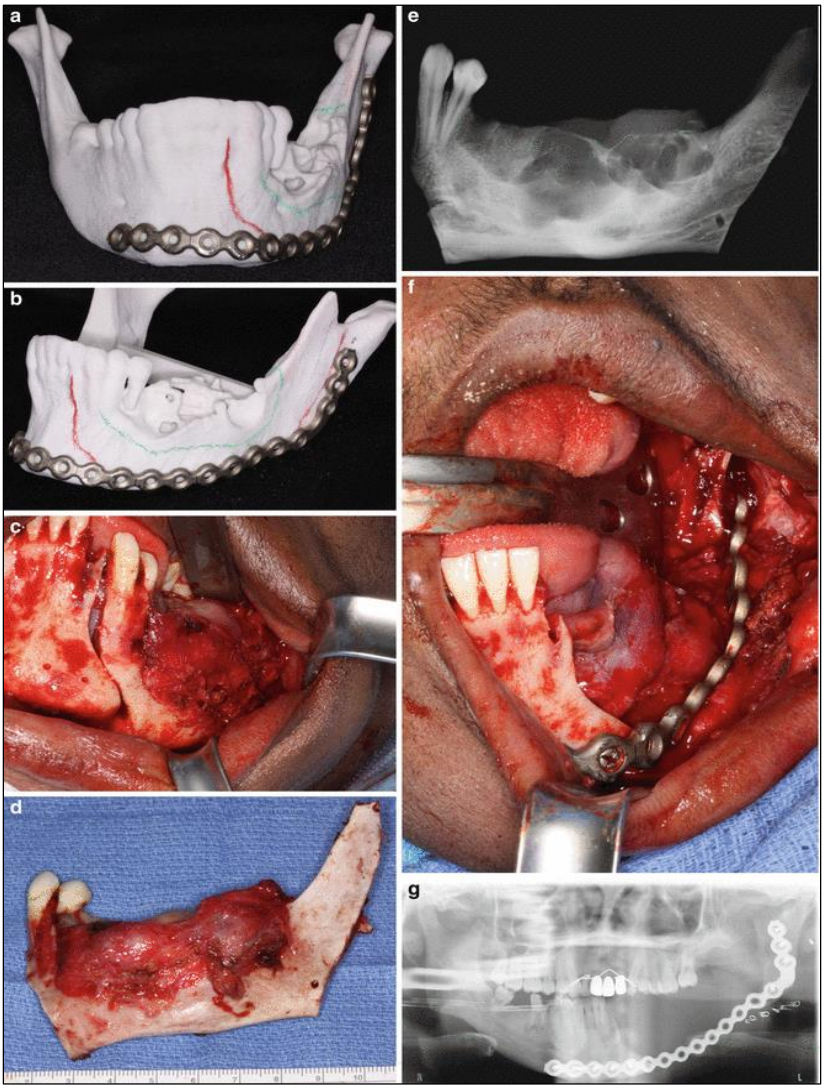

Fig 1: Clinical and radiographic image of conventional solid ameloblastoma case [7]

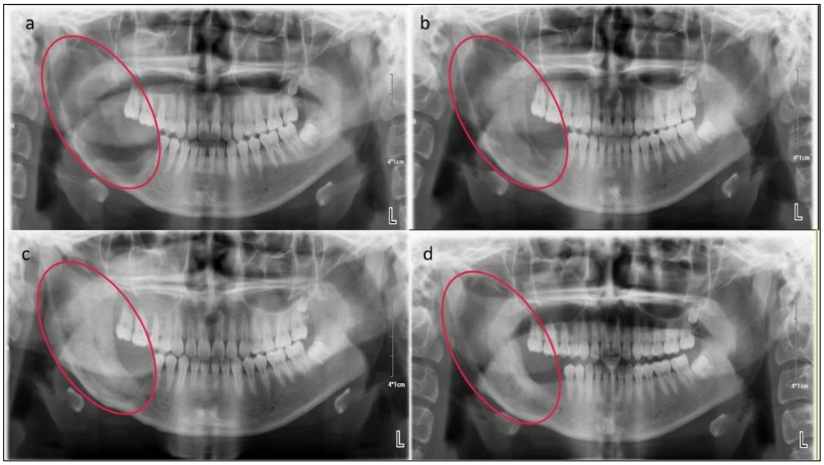

Fig 2: Panoramic film images of an ameloblastoma case during the treatment period. Preoperative and postoperative comparative panoramic image of patient undergoing the curettage combined with bone cavity opening. Preoperative panorama (a), postoperative 3 months (b), postoperative 6 months (c), postoperative 1 year (d) [8]

1.1.2 Unicystic ameloblastoma

Unicystic ameloblastoma, which constitutes 13% of all ameloblastoma cases, is a tumor different from other types of ameloblastoma in terms of its clinical, radiological, pathological features and response to treatment[4]. Unicystic ameloblastoma, whether originating spontaneously as a neoplasm or arising from the neoplastic transformation of non-neoplastic cyst epithelium, predominantly affects young patients in their second and third decades of life. Typically localized to the mandibular molar region, this tumor often remains asymptomatic, though large lesions can prompt painless swelling in the jaws. Clinically, radiographically, and macroscopically, these cysts closely resemble dentigerous cysts. Radiologically, unicystic ameloblastoma commonly exhibits a radiolucent appearance encircling the crown of the impacted mandibular third molar, mirroring that of a dentigerous cyst. Accurate diagnosis relies heavily on microscopic examination of the lesion material, as clinical and radiological findings often mimic those of odontogenic cysts. Treatment typically involves enucleation, akin to cyst management [6].

1.1.3 Non-osseous peripheral ameloblastoma

Peripheral ameloblastoma, which accounts for only 1% of cases, is believed to originate from epithelial remnants beneath the oral mucosa or basal epithelial cells of the surface epithelium. Typically painless and non-ulcerated,

this lesion on the gingival or alveolar mucosa is commonly found in the mandible and posterior alveolar mucosa, and it typically responds favorably to local surgical removal [4].

1.1.4 Metastasizing ameloblastoma

Although benign tumors typically do not metastasize, certain benign tumors such as pleomorphic adenoma, tuberous sclerosis, and giant cell bone tumor have been documented to spread to distant locations. Ameloblastoma, despite its benign nature on histology, is known for its propensity to metastasize, leading to the designation of metastasizing ameloblastoma (METAM). This condition, characterized by the spread of an otherwise benign odontogenic tumor, is rare and challenging to diagnose. The diagnosis of METAM relies on the presence of histological features consistent with benign ameloblastoma in both primary and secondary lesions. However, predicting metastasis based on histology alone is not feasible. Management of METAM involves a complex treatment approach, including aggressive resection of metastatic lesions alongside thorough curettage of the primary tumor site [9].

1.2 Calcified epithelial odontogenic tumor (Pindborg tumor)

The calcified epithelial odontogenic tumor, representing less than 1% of odontogenic tumors, is presumed to originate from the enamel epithelium of an impacted tooth. Characterized by cells closely resembling those of the tooth enamel's stratum intermedium, it occurs with equal frequency in both genders and is most commonly observed in individuals aged between their 3rd and 5th decades [10]. Predominantly affecting the mandible, particularly the molar region, this tumor typically manifests as an asymptomatic, slowly enlarging swelling. Radiographically, it presents as either unilocular or multilocular radiolucent lesions with borders resembling a comb's indentation. Despite sharing biological characteristics with ameloblastoma, the calcified epithelial odontogenic tumor is generally slow-growing and minimally aggressive. Treatment typically involves local resection, which includes limited excision of the surrounding bone [11].

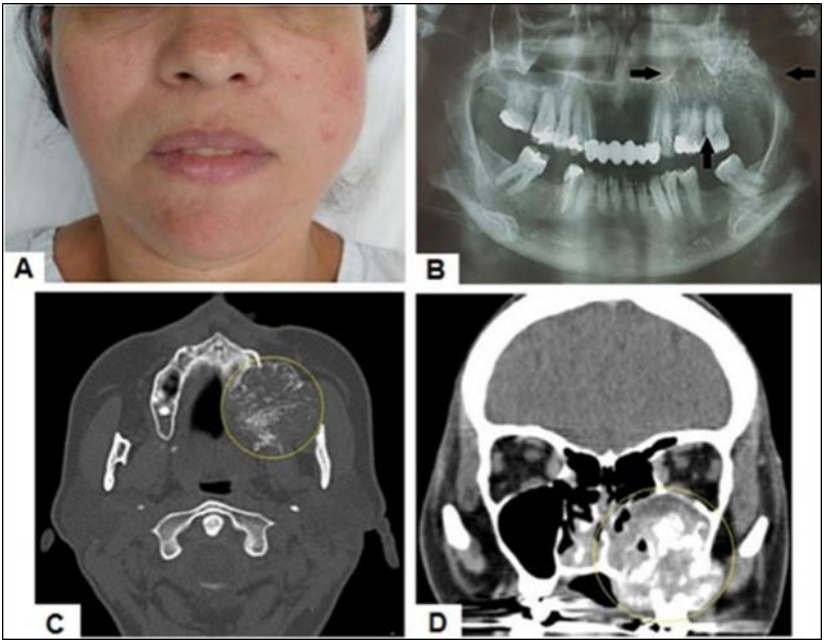

Fig 3: Clinical and radiographic image of calcified epithelial odontogenic tumor (Pindborg tumor) [12]

1.3 Squamous odontogenic tumor

The squamous odontogenic tumor, a rare benign odontogenic tumor, is believed to originate from epithelial remnants. This tumor displays no gender predilection and can occur at any age. Equally prevalent in the maxilla and mandible, it tends to manifest in the maxilla, particularly in the incisor-canine region, and in the mandible, typically in the canine-molar region or on the lateral aspects of the teeth [13]. Clinical signs may include tooth mobility, sensitivity to percussion, and a painless to mildly painful gingival swelling. Radiographically, the squamous odontogenic tumor appears as a semicircular or irregular triangular radiolucent defect adjacent to the root of the involved tooth. Occasionally, the borders of this defect may exhibit a sclerotic rim, resulting in smoother margins. Conservative local excision and curettage are typically the preferred treatment modalities, with low recurrence rates reported following excision [14].

1.4 Clear cell odontogenic tumor

The clear cell odontogenic tumor, a rare entity with uncertain histogenesis, is believed to originate from odontogenic tissues. Characterized by distinctive clear cells resembling glycogen-rich presecretory ameloblasts,

this tumor typically presents in individuals in their fifth decade of life, with no predilection for location between the maxilla and mandible[15]. Radiographically, it often appears as unilocular or multilocular radiolucent lesions. However, despite its rarity, the clear cell odontogenic tumor tends to exhibit aggressive behavior clinically, invading adjacent tissues and showing a propensity for recurrence. Even after radical surgical intervention, relapse remains a common occurrence in the majority of cases [16].

2. Tumors arising from odontogenic ectomesenchyme

2.1 Cementoblastoma

Cementoblastoma, while occurring equally in both genders and across various age groups, predominantly affects individuals under 25, with a threefold higher incidence in the mandible compared to the maxilla, particularly around the mandibular first molar. It's essential to differentiate it from cementomas, as cementoblastoma involves proliferating tissue mass contiguous with tooth root cementum, typically resulting in a bulb-shaped appearance [5]. Unlike cementomas, where the cementum separates from the tooth root, cementoblastoma maintains its attachment. Usually asymptomatic, cementoblastoma presents as a slow-growing lesion often causing cortical bone expansion. Radiographically, it's characterized by a round radiopaque mass encircled by a uniform radiolucent rim. Treatment typically involves surgical removal of the affected tooth along with the calcified mass, although root amputation and endodontic therapy may be considered. With total excision, the prognosis is excellent, with no reported instances of recurrence [15].

Fig 4: Radiographic image of cementoblastoma case [17]

2.2 Odontogenic myxom

Odontogenic myxoma, arising from the mesenchymal component of tooth germ tissues like the dental ligament, papilla, and follicle, is typically observed during the second and third decades of life. These lesions often lack distinctive features, and smaller ones may be asymptomatic, detectable only through routine radiographic screening. As odontogenic myxomas grow, they can lead to jaw expansion and cortical destruction, primarily affecting the mandibular premolar to molar regions and extending into the maxilla, involving the sinus and zygoma. Maxillary sinus involvement can cause sinus wall displacement and exophthalmos [18]. Frequently associated with impacted or non-impacted teeth, odontogenic myxomas appear as multilocular radiolucent defects on radiographs, resembling a honeycomb or tennis racket. While they can erode bone, adjacent tooth root resorption is rare. Surgical excision is the standard treatment, with larger lesions requiring more extensive resection. Despite their benign nature, odontogenic myxomas' lack of encapsulation increases the risk of recurrence, occurring in about 25% of cases; however, the overall prognosis remains favorable [19].

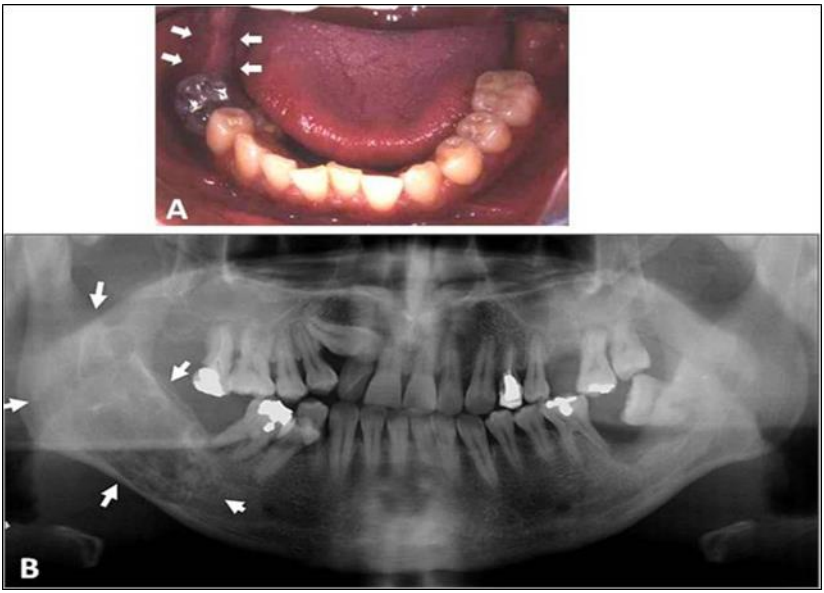

Fig 5: Clinical and radiographic image of odontogenic myxoma case [20]

2.3 Odontogenic fibroma

Odontogenic fibroma, which consists of fibrous tissue containing cementum and odontogenic epithelial clusters, has central, granular cell and

peripheral types. Central odontogenic fibroma is one of the rare odontogenic tumors that occur only in the jaw bones. It is a type of tumor that is painless, grows slowly and can expand. It is common in the maxilla and maxillary anterior and first molar region. Many lesions are surrounded by a sclerotic line. Small odontogenic fibromas are unilocular lesions with regular borders [21, 22]. These lesions appear in the apical region of an erupted tooth. Larger odontogenic fibromas give a multilocular radiolucent appearance. Granular cell odontogenic fibroma occurs frequently in the molar region of the maxilla and mandible. Although they often do not cause symptoms, they can sometimes cause a painless expansion in the affected area. The lesion appears as a regularly circumscribed, unilocular radiolucent area and may contain small calcifications. Peripheral Odontogenic Fibroma is the soft tissue form of central "intraosseous" odontogenic fibroma. This uncommon gingival lesion is soft and vascular in nature when it first occurs. Later, it is usually seen as a pale red-pink colored, hard, slow-growing lesion, usually sessile, seated in the gingival tissue and covered with normal-looking mucosa. In some cases, calcification may be seen in the area where the tumor occurs. The lesion has no relationship with the underlying bone. All three types of odontogenic fibromas are usually treated by enucleation and curettage. Relapse is rare and the prognosis after treatment is quite good [22].

3. Mixed tumors originating from odontogenic ectomesenchyme and odontogenic epithelium

3.1 Odontoma

Odontoma is a term that describes the growth of functional ameloblasts and odontoblasts that form enamel and dentin, in which both epithelial and mesenchyme cells differentiate together. Odontomas are considered a developmental anomaly (hamartoma) rather than a true neoplasia. Odontomas that have completed their development contain largely enamel, dentin and some pulp and cementum tissue. Although the etiology of odontoma is unknown, it is suggested that local trauma or infection plays a role in the emergence of this lesion. Odontomas are the most common of all odontogenic tumors, and the prevalence of odontoma exceeds that of all other odontogenic tumors combined [23]. Odontomas are divided into two: compound odontoma and complex odontoma. Compound odontoma contains numerous small tooth-like structures organized by enamel, dentin, pulp and cementum tissues during odontogenesis. Complex odontoma, on the other hand, occurs during odontogenesis when enamel, dentin, pulp and cementum tissues come together in an unorganized manner, creating an irregular structure that does not resemble a tooth [23, 24]. These lesions contain radiopacities containing clumped

irregular enamel and dentin that do not resemble tooth structures anatomically. Most odontomas occur in the first two decades of life. Odontomas are more common in the maxilla than the mandible. The radiographic appearance of odontomas is characteristic, and since most lesions are asymptomatic, they are usually detected during routine radiographic examinations. Compound odontoma is like a cluster of various tooth-like structures. These tooth-like structures are surrounded by a narrow radiolucent area. Complex odontoma gives the appearance of a calcified mass in the radiodensity of the tooth structures surrounded by a radiolucent line. Treatment of odonotomas is local excision, and these lesions do not show recurrence after surgical removal. These lesions, which have a very good prognosis, require attention because they are radiographically very similar to both odontoameloblastoma and ameloblastic fibro odontoma. For this reason, it is important to undergo microscopic examination of all odontomas [23, 24].

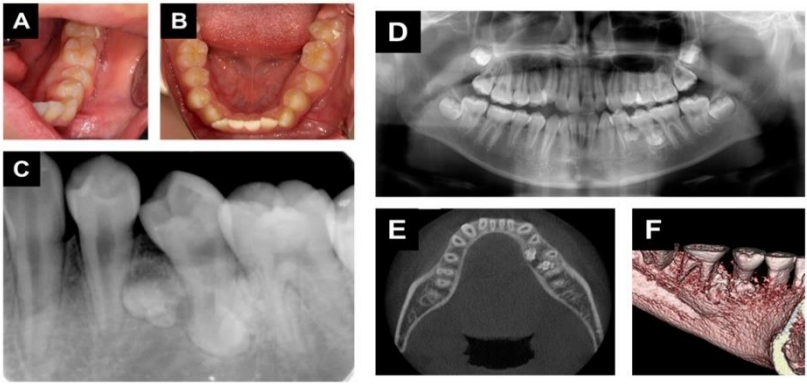

Fig 6: Compaund odontoma [25]

Fig 7: Complex odontoma [26]

3.2 Adenomatoid odontogenic tumor

Adenomatoid odontogenic tumor is a tumor originating from the enamel epithelium. Adenomatoid odontogenic tumor is classified together with epithelial odontogenic tumors according to some sources. However, the fact that the tumor most often consists of dentinoid material (rarely enamel matrix) suggests that it is an epithelial tumor induced by odontogenic ectomesenchyme. Adenomatoid odontogenic tumor is more common in young patients. Adenomatoid odontogenic tumor, which is twice as common in women than in men, is three times more common in the maxilla than in the mandible [27]. Adenomatoid odontogenic tumors are often asymptomatic, but lesions that reach large sizes cause painless swelling in the bone. In the majority of cases, the tumor appears as a radiolucent area involving the crown of an impacted tooth (usually a canine). Adenomatoid odontogenic tumor is often completely radiolucent, but sometimes the tumor may contain small calcifications (snowflake) on radiographs. Since adenomatoid odontogenic tumor is a benign lesion, it is very easy to enucleate it from the bone. They do not have aggressive behavior. The prognosis is very good and recurrence after enucleation is very rare [28].

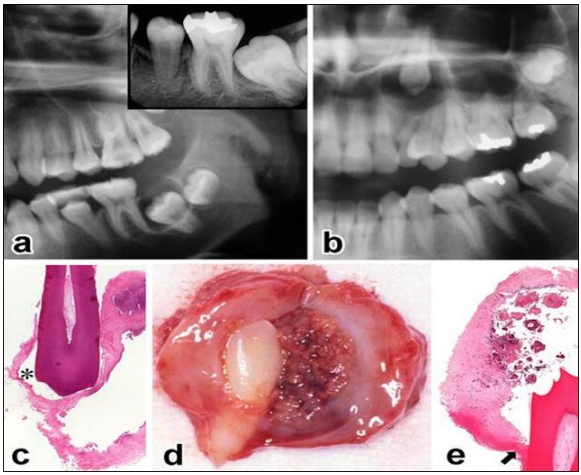

Fig 8: Clinical and radiographic image of adenomatoid odontogenic tumor case [29]

3.3 Ameloblastic fibroma

Ameloblastic fibroma is a mixed tumor of odontogenic origin, characterized by spontaneous neoplastic proliferation of both epithelial and mesenchymal tissues before enamel or dentin formation occurs. Most cases of ameloblastic fibroma occur in the second decade of life and are more common in women than men. Ameloblastic fibroma often occurs in the mandibular

molar region, like ameloblastoma [30]. Ameloblastic fibromas are often asymptomatic and can only be detected on radiographs taken for another purpose. The slow-growing lesion, which does not tend to infiltrate the trabecular bone, may cause moderate swelling, pain, and sometimes tenderness in the area where the tumor occurs. Ameloblastic fibromas can appear radiographically as unilocular or multilocular radiolucent. The borders of the lesion may be surrounded by a sclerotic line and are smooth [31]. Previously believed to be a harmless lesion, ameloblastic fibroma is now reported to have a significant risk of recurrence after local excision or curettage. Some of these problems may be related to the inclusion of small lesions such as odontomas that develop between neoplastic ameloblastic fibromas. Recurrence occurs in approximately 20% of conservatively removed ameloblastic fibromas. Some researchers say that lesions that recur after excision behave more aggressively. Approximately 50% of ameloblastic fibrosarcomas occur as a result of recurrence of ameloblastic fibromas [32].

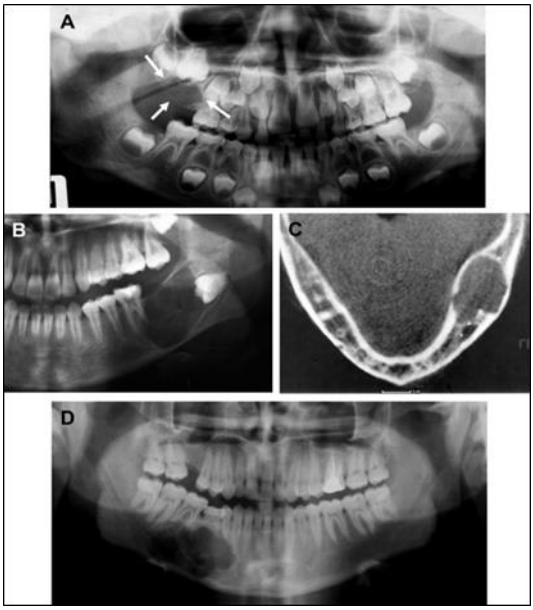

Fig 9: Radiographic image of ameloblastic fibroma case [33]

3.4 Ameloblastic fibro-odontoma

Ameloblastic fibro-odontoma, which resembles ameloblastic fibroma in general features, differs from ameloblastic fibroma in that it contains enamel and dentin. Ameloblastic fibro-odontoma occurs mostly in children. 10 years is considered the average age. The tumor, which is rare in adults, is seen

equally in the maxilla and mandible. Some lesions occurring in the maxilla can affect the maxillary sinus [32]. The most common clinical findings related to ameloblastic fibro-odontoma are painless swelling in the bone and failure of the tooth in that area to erupt. Radiographically, in most cases, a unilocular or sometimes multilocular radiolucent appearance with an unerupted tooth inside or on its edge, regularly circumscribed, is observed. Inside this radiolucent image, there are calcified structures similar to the radiodensity of tooth structures. Because the lesion is easily separated from its place in the bone, ameloblastic fibro-odontoma is usually treated with conservative curettage. The prognosis is very good. Recurrence is uncommon after conservative removal [32, 34].

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Chapter - 3
Phytotherapeutics in Periodontics

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Chapter - 3

Phytotherapeutics in Periodontics

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Abstract

From the ancient times, plants and plant based products are used for treating numerous diseases as a traditional method of treatment. As per the saying "Food is medicine", the increasing use of these herbs in the form of food are commonly used for treating common ailments. Nowadays, the use of herbal products called as phytotherapeutics are gaining interest in dentistry because of their natural activity, less side effects and cost effectiveness when compared to conventional drugs. Additionally, these traditional medicines were found useful in curing various oral diseases. Of all oral infection, gingival and periodontal diseases appear to be the most commonest diseases of oral cavity. As herbal drugs exhibit properties like antimicrobial, anti-inflammatory, antioxidant, antiplaque activities they aid in improving periodontal health. This chapter highlights the effects of herbs on gingival and periodontal disease management and the various types of herbs which can be used in the different forms like mouthwash, oral gel, local drug delivery, dentifrices and as adjunct to many treatment forms. Modern treatment focuses on alternative approach and numerous researches are going nowadays as a futuristic approach.

Keywords: Phytotherapeutics, herbs, oral health, periodontal disease**,** phytochemicals

1. Introduction

Oral health and its maintenance are essential for preventing oral diseases and their harmful consequences which occur due to poor oral hygiene posing a substantial burden on people worldwide. The demographic shift and ageing of the population are the prime cause for various chronic diseases which have significantly affected the public health [1, 2]. The study by Global Burden of Disease 2010 has reported that diseases of oral cavity contributed to 15 million disability-adjusted life years, signifying people affected averagely with disease as 224 years per 100,000 populations [3]. World

Health Organization (WHO) has stated that diseases of the oral cavity was found to stand out as a global problem and estimated to affect worldwide about 3.5 billion people [4]. The oral health is most commonly compromised by dental caries, periodontal diseases, loss of teeth, dry mouth, lips and mouth cancers [5]. Out of these, periodontal diseases stand out as the most happening conditions in the mouth. Only few people were found to have a healthy oral cavity with the dental diseases being prevalent among 3.5 billion people globally, as per WHO.

2. Periodontal diseases and phytotherapeutics

Since the dawn of history, Gingival and periodontal diseases have affected human beings. In paleopathology, studies have shown bone loss as evidence for destructive periodontal disease which have afflicted early humans. During the ancient Greek era, Hippocrates of Cos (460-377 B.C.) revealed that formation of 'pituita' or calculus with gingival bleeding causes inflammation of gum [6].

The starting stage of periodontal diseases is Gingivitis represented by clinical signs of gingival inflammation having dental plaque as a primary etiological factor. It affects only gingiva and shows no attachment loss. Other etiology are systemic diseases, non-plaque induced factors (bacteria, virus etc.) and certain medications [7]. Whereas periodontitis is the progression of gingivitis which is chronic, multi-factorial, host-mediated infectious disease causing destruction of tooth supporting tissues [8].

Various researches have caused evolution of many theories regarding etiology, diagnosis, management and prognosis of periodontal diseases [9]. Dental Plaque and subgingival microbial flora remain as the main cause for periodontitis. The progression of periodontal diseases is mainly depended upon poor oral hygiene and dental plaque accumulation [10]. In the recent times, there is an astonishing improvement in the usage of herbs and herbal medicines for treating varied type of ailments. Due to high incidence of new diseases and re-emergence of many infectious oral diseases there is a major concern among people now-a-days. The necessity for discovering new antimicrobial substance from natural sources like plants and traditional herbal medicine have aroused because of the emerging multiple drug resistance to various oral microbes. The method of using herbs or plants for treating health ailments was called "Phytotherapeutics", a Latin term in which "phyto" denotes plant and "therapy" denoted healing and cure. These agents act as an excellent supportive therapy for managing periodontal diseases [11].

Phytotherapeutic agents include all herbs, their preparations and medicines prepared from them containing the biologically active ingredient of the plants or parts of plant in order to obtain their therapeutic benefits [30]. It is a known fact from our olden days, where our ancestors used to tell about their secret concoctions to heal any ailments. The practice of massage of gingiva is common among Sumerians, the Babylonians and the Assyrians along with multiple herbal medications for any diseases of oral cavity [12]. Among the global population 80% use plant based products for their primary oral health care like teas, extracts and other bioactive preaparations, estimated to be US$ 50 billion per year [13]. The conventional antibiotics are known to cause detrimental adverse effects which can be tackled by using the herbs with active biological active ingredients through phytotherapeutic approach. These bioactive compounds like alkaloids, terpenoids, tannins and flavonoids have been researched *in vitro* for their therapeutic effects and antimicrobial properties.

People nowadays show more interest in herbal products due to their affordability with higher safety margins, making these herbal based oral therapy as an effective alternative medicine. Furthermore, the commonly used drugs are known to cause various reactions like hypersensitivity, immune suppression, and allergic reactions and its constant use lead to resistance to antibiotics. Hence, the use of herbal products as dietary supplements has increased nowadays in order to tackle systemic and oral diseases [14].

3. Types of phytotherapeutic agents and their use in managing periodontal diseases

3.1 Curcumin (*Curcuma longa*)

Curcumin, traditionally known as 'turmeric' belongs to family Zingiberaceae, being a rhizomatous herbaceous perennial plant. It grows in tropical area of South Asia between temperatures of 20 °C and 30 °C. It is a spice based plant being orange, oblong tubers which is dried and turned into a yellow powder. It is slightly acrid, bitter at the same time has sweet taste. Turmeric is extensively used and studied for its effect on oral diseases due to their antimicrobial, astringent, anti-inflammatory, antiseptic, antioxidant, anti-carcinogenic and other beneficial properties [15].

It has polyphenol curcumin in its rhizome. Curcumin contains curcuminoids namely curcumin (diferuloylmethane), demethoxycurcumin, and bisdemethoxycurcumin. The most biologically active constituent is curcumin, containing 0.3-5.4% of raw turmeric. From the ancient ayurvedic

medicine ages, Curcumin has been utilized extensively as it is not toxic and has a varied curable benefits [16].

Curcumin exhibits potent anti-inflammatory effects which are explained based on its various mechanisms and its action on various molecular targets. The transcription factor nuclear factor kappa-B (NF-κB) is downregulated by curcumin, a key signaling molecule in initiating inflammation [17] as well as in cell multiplication, oncogenesis, and cellular modification [18]. It also suppresses the cyclooxygenase-2 expression, an enzyme required for synthesis of prostaglandins which is responsible for causing inflammation, including periodontitis. At the same time curcumin antagonizes nitric oxide synthase production, a pro-inflammatory molecule regulated by NF-κB and induces degradation [19]. Curcumin acts against both bacteria [20] and fungi [21]. It acts as bacteriostatic [22] as well as bactericidal for both Gram-positive bacteria [23] and Gram-negative bacteria.

The antibacterial properties of curcumin have been explained through various mechanism of action. The bacterial cell division is known to be inhibited by curcumin [23] by triggering membrane permeabilization of bacteria, thus causing permanent damage and cellular death in both Gram-positive and Gram-negative bacteria [24]. Curcumin down regulates Streptococcus mutans biofilm formation by inhibiting sortase A enzyme which causes bacterial attachment [25] to tooth surfaces [26]. Various studies revealed that curcumin showed additional antibacterial effects along with various antibiotics such as cefixime, vancomycin, and tetracycline against P. aeruginosa and S. aureus by suppressing their virulence, plaque initiation and quorum sensing [27].

3.2 Guava (*Psidium guajava*)

The Guava has many phytoconstituents such as flavonoids, tannins, phenolic compounds, essential oils, sesquiterpene alcohols, triterpenoid acids and vitamins. All these constituents exhibit various effects such as anti-inflammatory, antimicrobial, antispasmodic, analgesic, anticancer, antioxidant, antihyperglycemic and hepatoprotective actions. Guava is primarily composed of Vitamin C (Ascorbic acid), an excellent antioxidant causing fibroblast differentiation alteration and induce collagen formation by modulating procollagen gene expression. Vitamin C aids in wound healing by acting along with bioflavonoids [28].

Quercetin, one of the flavanoid in guava acts against periodontal pathogens *Aggregatibacter actinomycetemcomitans* (Aa), *Porphyromonas gingivalis* (Pg), *Prevotella intermedia* (Pi), and *Fusobacterium nucleatum*

(Fn). Other constituent exhibiting antibacterial properties is tannin which is present in bark [29].

Essential oils and decotion from guava leaves reduce free radicals by scavenging hydrogen peroxide and superoxide anion. The guava leaf extracts are used for treating bleeding gums in the gargle form, while the mouthwash can be prepared from guava roots and bark extract [30]. Traditionally, toothpaste is made from the tender leaves of guava which can be a used for maintaining oral health.

Guava exhibits anti-inflammatory property by suppressing prostaglandin (PG), kinin, and histamine [31]. It has the ability to alter immune activity by acting on NF-kβ [32] and inhibit lipopolysaccharide induced NF-kβ activation [33]. Blocking NF-kβ will help in preventing resorption of bone as a result of inflammation in periodontal disease [34].

3.3 Neem (*Azadirachata indica*)

In India and South Asia, *Azadirachata indica* commonly known as Neem is considered to be one of the oral hygiene aid for the past 1000 yrs. Phytocomponents present in neem are Nimbin, azadirachtin, nimbidin, epicatechin, nimbolide, catechin, gallic acid, and margolone. *Azadirachta indica* is a Meliaceae tree seen most commonly in Africa and Asia. Indigenous systems of medicine made use of all tree parts for treating various diseases, particularly against bacterial and fungal infections. Traditionally, neem leaves and seeds were taken after a meal and neem twigs were used for tooth brushing. It has been used as an antiseptic and astringent. Neem has antiviral, antifungal, antibacterial, antiinflammatory, anticariogenic, antipyretic, analgesic, antihelminthic, and anti-oxidant.

Neem leaf extract has found to exhibit strong action against hyperglycemia and *in vitro* and *in vivo* on animals [35]. It has high potency in suppressing plaque accumulation and for treating other oral infections [36]. It was also evaluated for its broad antibacterial activity [37]. Recent studies have elaborated the use of neem in treating gingivitis, periodontitis and dental caries.

3.4 Aloe vera (*Aloe barbadensis*)

The *Aloe barbadensis* traditionally called Aloe vera, belong to the Lily family. It is a xerophyte variety plant as it can survive with less or erratic water because of its ability to retain large amount of water. It is rich in vitamins, minerals, enzymes, fatty acids, sugars, amino and salicylic acids. Natural anthraquinones with antimicrobial properties present in aloe vera are

Aloin, barbaloin, Aloe emodin, chrysophanic acid, isobarbaloin, aloetic acid, anthracine, ethereal oil, cinnamonic acid esters, anthranol & resistannol [38]. Numerous studies demonstrated the various biological actions of Aloe vera which include anti-inflammatory, antibacterial antiviral, antioxidant, and antifungal actions [39].

Aloe vera mouth rinse showed its efficacy in reducing plaque formation and treating gingivitis. Aloe vera contains polysaccharides which is responsible for antibacterial activity via phagocytic leukocyte activation. It neutralizes the free oxygen radical's production by alternative and chemical pathways of complement activity [40]. Aloe vera aids in curing various ailments by its numerous healing properties. It is commonly used as dietary supplement. Due to its powerful antioxidant activity and accelerator of wound healing, useful after periodontal surgery and for treating lesions of gingival caused by trauma from toothpick, toothbrush or solid food.

3.5 Tulsi (*Ocimum sanctum*)

Tulsi is an *Ocimum sanctum* Linn plant which comes under Labiatae family. It has a square stem with specific aroma. It is widely present in India, Australia, Malaysia, West Africa and some of the Arab countries. It is known for its highly complex chemical constituents which gives biological benefits. Tulsi possess anti-bacterial especially ES. Mutans, *E. coli* and *S. aureus* [41]. Other effects of tulsi are ant cariogenic, anti-ulcerogenic properties, anti-fungal and anti-viral activity.

The tulsi leaves contain essential oils including eugenol, germarene-A, clemene, caryophyllene, and caryophyllene oxide all exhibiting antibacterial activity. Other phytochemicals present in them are ursolic acid, rosmarinic acid, and oleanolic acid. All these constituents show activity against Gram-positive and Gram-negative bacteria [42]. They exhibit antibacterial effect by degrading their cytoplasmic membrane and stimulating cellular potassium release. These mechanisms were found to be related to their action against the periodontal microbe in plaque namely *A. actinomycetemcomitans*.

Tulsi also known to possess immunomodulatory effect by stimulating interleukin-4, interferon and T helper cells thereby boosting the response of host to infection [43].

3.6 Pineapple (*Ananas comosus*)

Ananas comosus, a tropical fruit commonly called "pineapple" highly rich in vitamins, antioxidants and enzymes. It belongs to the bromeliaceae family. An elementary extract from pineapple, Bromelain is known to have

therapeutic qualities and shows antiedematous, anti-inflammatory, fibrinolytic and antithrombotic activities. Bromelain is equivalent to non-steroidal anti-inflammatory agents, glucocorticoids, and immunomodulators in their action. It acts against various aerobic and anaerobic microorganisms like *Porphyromonas gingivalis*, *Streptococcus mutans*, *A. Actinomycemcomitans*, *Escherichia coli* [44]. It reduces adverse side effects usually caused by antibiotic toxicity because of its increased body absorption which aids in better distribution in the tissues [45].

3.7 Tea tree oil (Melaleuca oil)

Tea tree oil, an essential oil with biological name Melaleuca oil used over years in Australia. From the ancient times, primary tree tea oil was particularly used for its antiseptic, anti-viral, anti-inflammatory and clinical efficiency. The phytoconstituent named terpenoids in tree tea oil is responsible for its therapeutic effects [46]. Tea tree oil prepared by steam distillation from the leaves of Melaleuca alternifolia, an Australian plant. The term tea tree oil was introduced as its leaves were traditionally used as tea substitute.

Tea tree oil is known to exhibit antimicrobial property against streptococci mutans and other oral pathogens. Certain studies have shown that tea tree oil can substitute chlorhexidine in its action [47].

3.8 Pomegranate (*Punica granatum*)

Pomegranate belongs to the family Punicaceae meaning Apple (“Pomum”) and seeded (“granatus”). The pharmacologically active constituents of pomegranate are ellagitannins, anthocyanins, flavonoids, punicic acid, anthocyanidins, and estrogenic flavones. Pomegranate peel possess antimutagenic, anti-inflammatory and antifungal activity. Pomegranate juice contains tannins which shows anti-oxidative, anti-atherosclerotic, antihypertensive and anti-aging properties [48].

Punicic acid is one of the prime constituent of pomegranate which shows good anti-inflammatory property by suppressing PG production. It reduces the NF-κB activity thereby preventing inflammatory periodontal disease [49]. Pomegranate extract can induce angiogenesis, fibroblast migration and proliferation and collagen production thus helping in fastening the wound healing.

3.9 Green tea (*Camellia sinensis*)

Camelia sinensis, comes under the theaceae family, a strong antioxidant with strong healing potential. Green tea is prepared solely from Camellia

sinensis leaves with minimal oxidation during processing. Various methods are used in processing and growing them to produce the many green tea varieties. Due to this processing, major quantities of polyphones and antioxidants are retained, giving maximum health benefits. The chief constituents of the leaf extracts are catechins like epicatechin, epigallocatechin gallate, epigallocatechin, and epicatechin gallate [51].

Catechins possess anti-oxidant and anti-inflammatory property. It helps in tackling inflammation thus reducing gingivitis. Green tea can reduce resorption of alveolar bone by decreasing the inflammatory cytokines and TRAP-positive multinucleated osteoclasts thus resulting in periodontal inflammation inhibition. The green tea gel when used as an adjunctive topical therapy for 4 weeks daily found to suppress inflammation [52].

3.10 Mango leaf (*Mangifera indica*)

Main constituent of Mango leaves are mangiferin along with anthocyanins, ascorbic and phenolic acids which contribute to antibacterial activity. It was used for brushing and also in the form of tooth powders. Only mature leaves majorily contain mangiferin and used for toothbrushing. Mangiferin (2C, Beta D-Glucopyranosyll, 3, 6, 7 tetra hydroxylxanthone) proved to have anti-inflammatory, cholerectic, diuretic and cardiotonic activity [53].

3.11 Garlic (*Allium sativum*)

Garlic primarily has sulfur-containing and other phytoconstituents. They express antioxidant and other biological activity. The compound with antibacterial effect in garlic is due to the oxygenated sulphur compound called allicin, an thio-2-propenesulfinic acid sallyl ester. This allicin is absent in raw garlic. The allicin is works by interacting with thiol containing enzymes, namely cysteine proteases and alcohol dehydrogenases [54]. The allicin found to reduce diverse thiol dependent enzymatic action which accounts for its wide antibacterial effect. The extract of garlic has strong antibacterial action. At the same time, it is thermo-labile, which when heated, forms various disulfide compounds [55].

Aqueous extracts of *Allivum sativum* possess best anti-microbial activity against gram positive and gram negative bacteria (Salmonella, Clostridium Staphylococcus, Klebsiella, Escherichia, Proteus, and Mycobacterium) and antifungal activity particularly against Candida albicans, also antiparasitic activity and antiviral activity [54]. The extracts of garlic could be a viable periodontitis therapy because of its superior efficacy and powerful action against bacteria *P. gingivalis* and *A.*

actinomycetemcomitans. Garlic extract exhibited inhibitory action on Staphylococcus epidermidis even at sub-MIC levels, thus preventing plaque formation [56].

3.12 Clove (*Syzygium aromaticum*)

Clove, a potent spice commonly used from olden days in the food industry and for many therapeutic purposes. Clove Oil which are phenols is highly volatile (16-21%), other constituents being Eugenol (80-88)%, acetyl eugenol (10-15) Pyrogallol tannins (10-13%), á and â-Caryophyllene, Methyl furfural and dimethyl furfural are main constituents. Clove oil is traditionally used an oral hygiene aid. It is known for its germicidal activity and hence found useful in curing painful tooth, mouth ulcers and sore gums.

Clove oil mainly constitutes eugenol, which is one of the commonly used substances in dentistry for a longtime [57]. The extract of clove oil has action against *Actinobacillus* Sp. The aqueous extract showed effective zone of inhibition than acetone extract which is an *in vitro* antibacterial study [58]. Antioxidant polyphenolic substances in clove namely kaempferol and myricetin showed bacteriostatic action against periodontal pathogens.

3.13 Lemon grass oil (*Cymbopogon citrates*)

Lemon grass oil is a perennial aromatic grass which is fast growing. It is obtained by extraction from Cymbopogon citrates. The therapeutic properties of lemon grass oil are antimicrobial, anti-inflammatory, antiseptic [59]. *C. citratus* possess constituents such as geraniol, neral, citral, limonene, linalool, citronellal, terpineol and dipeptene. The major component of these is vitral for around 65-85% [60].

Some studies have elaborated the effects of thiol antioxidant present in lemongrass oil mouthwash on gingivitis patients. They exhibited decrease in the antioxidant levels before treatment and subsequent increase in their levels after the non-surgical treatment. Hence they can act as an effective adjunct to nonsurgical periodontal therapy [61].

3.14 Propolis

It is a resin from trees of poplars and conifers or from flowers of genera clusia which are collected by bees. Natural pharmacological substances present in propolis are flavonoids, aromatics and phenolics. The anti-inflammatory components in propolis are Caffeic acid and phenethyl ester (CAPE). Propolis has a broad spectrum of biological properties like antimicrobial, anti-inflammatory, antioxidant, anaesthetic and cytotoxic properties [62]. It helps to maintain periodontal ligament cells viability as a storage media for avulsed teeth [63].

Brazilian propolis is one of the variant of propolis also known as green propolis. It is well known for its biological and chemical properties. Similar to other propolis, it exhibits antioxidant, anti-inflammatory, anticarcinogenic and antiparasitic effects. It is an integral part of folk medicine in Brazil, were it is a day-day practice for people for using propolis for daily tooth brushing and mouth rinsing.

3.15 Triphala

Triphala as the name suggests is a combination of herb containg three fruits originating in India. Traditionally used as a complete body cleanser, it is well known in ancient Indian folklore. It is extensively used in Indian Ayurvedic medicine as a natural healer.

Triphala constitutes fruits namely *Terminalia chebula* (chebulic myrobalan), *Terminalia bellirica* (beleric myrobalan), *Phyllantus emblica* (emblic myrobalan). Their valuable effects include antibacterial, anti-viral, anti-inflammatory, antihyperglycaemic, anti-oxidant, antihypertensive, anti-carcinogenic and anti-collagenase activity [64]. Triphala is found to have strong inhibitory action on PMN-type matrix metalloproteinase (MMP-9) seen in chronic periodontitis patients when assessed with an herbal drug (kamillosan), and doxycycline, known for its inhibitory activity MMP-9. Triphala mouthwash helps in treating bleeding gums, gingivitis and suppressing plaque formation. Recent studies focuses on elaborating its anti-collagenase activity against periodontal diseases.

3.16 Chamomile flowers (*Matricaria chamomilla*)

Chamomile flower comes under the Asteraceae family, which is rich in essential oils, flavonoid compounds and sesquiterpenes such as α-bisabolol and chamazulene. Conventionally, it is a herbal medicinal product used as an aid in treating minor oral and throat ulcers and inflammations [65]. Chamomile flower extract has anti-inflammatory and antioxidant properties topically [66]. The significant properties of chamomile made it to be used as an effective herb in periodontal disease management. Hans *et al*. [67] and Saderi *et al*. [68] explained the action of chamomile oil against *P. gingivalis*. The essential oil also blocks the formation of plaque on tooth surface usually caused by *A. actinomycetemcomitans* and *T. denticola*.

3.17 Noni

Noni also known as Indian Mulberry is a common traditional plant of the South Pacific region [69]. Their entire plant is used for the treatment of a numerous diseases. Today, Noni has evolved as an effective medicinal plant

due to its various properties like analgesic activity, immunomodulatory, antimicrobial, anticancer ad anti-tubercular effect. All of these properties make it to be used as a supplemental health drink [70, 71]. Noni possess phytochemicals and nutrients such as scopoletin, terpenoids, octoanoic acid, anthraquinones, alkaloids, β-sitosterol, flavone glycosides, carotene, linoleic acid, potassium, amino acids, vitamin A, vitamin C, calcium and phosphorus [72, 73].

Noni is found effective against gingivitis and the mouthwash made from ripe noni fruit extract exhibited improvement in gingival status because of its antibacterial, anti-inflammatory properties [74, 75]. Noni extracts were also found to enhance proliferation of cells, reduce proteolysis, matrix deposition and mineralization *in vitro* studies. It thus helps in periodontal tissue regeneration [76] and also possesses osteoinductive potential as it aids in differentiation of osteoblasts from the bone marrow stem cells and [77]. Hence, noni can be used for regenerating bone in periodontal disease bone defects [78].

4. Conclusion

Periodontal diseases stand out as a constant evolving disease due its inflammatory nature which differs according to the host response. The main aim of treating these diseases depend on improving he host immunity and preventing inflammatory response thus reducing periodontal tissue destruction. Herbal products can be effectively used for this purpose as it exhibits less side effects and enhanced health benefits. As per the saying by Hippocrates, “Let food be your medicine and let medicine be your food” still applies to this present era which signifies the fact that “we are what we eat”. Phytotherapeutics as an adjunct to periodontal treatment can work effectively. However, herbal products differ in their effectiveness; which necessitates the of use plant based preparations carefully and appropriately. The modern world focuses on alternative therapeutic approaches in treating enormous number of diseases nowadays. It is necessary to carry out more elaborative studies and long term researches utilize these herbal products in a possible way in managing periodontal diseases and to use it in daily clinical practice.

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Chapter - 4
Exploring Micro-Needling Potential in Dentistry: A Promising Adjunctive Therapy

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Chapter - 4

Exploring Micro-Needling Potential in Dentistry: A Promising Adjunctive Therapy

Dr. Anishka Dhanai and Dr. Neelam Das

Abstract

Microneedling, originally utilized in dermatology, has emerged as a promising technique within dentistry, particularly in periodontology. This minimally invasive procedure involves creating controlled micro-injuries to stimulate tissue regeneration and collagen production. In the realm of periodontal care, microneedling shows potential for enhancing gingival tissue regeneration, modifying gingival phenotype, and improving treatment outcomes. These abstract highlights the diverse applications of microneedling in periodontology, including gingival tissue regeneration, reduction of periodontal pocket depth, delivery of therapeutic agents, and management of oral mucosal disorders. Additionally, microneedling offers a minimally invasive alternative to traditional surgical interventions, potentially improving patient comfort and recovery. Further research is needed to fully explore the optimal protocols and effectiveness of microneedling in addressing various periodontal conditions and modifying gingival phenotype.

Keyword: Microneedling, collagen induction therapy, growth factors, platelet concentrates, phenotype

Introduction

Microneedling, though primarily associated with dermatology and cosmetic procedures, has also found application in the field of dentistry, particularly in the oral and maxillofacial surgery and periodontics. In dentistry, micro needling techniques are adapted to address specific oral health concerns and conditions affecting the gums, teeth, and surrounding tissues [1].

One notable application of micro needling in dentistry is in the treatment of gingival recession, a common condition where the gum tissue around the teeth recedes, exposing the tooth root. This recession can lead to aesthetic concerns and increased sensitivity. Microneedling can be employed to stimulate the growth of new collagen and elastin fibers in the gum tissue,

promoting tissue regeneration and potentially reversing gingival recession. Moreover, microneedling can assist in facilitating the absorption of topical medications or growth factors into the periodontal tissues. By creating micro-channels in the gum tissue, microneedling enhances the delivery and effectiveness of these therapeutic agents, aiding in the treatment of periodontal diseases such as gingivitis and periodontitis [2].

In oral surgery, microneedling techniques may be utilized to promote wound healing and tissue regeneration following procedures such as dental implant placement or extraction. The controlled micro-injuries induced by microneedling can accelerate the formation of new blood vessels and collagen fibers, supporting faster recovery and minimizing post-operative complications. Furthermore, microneedling has shown promise in the management of oral mucosal disorders, such as lichen planus and recurrent aphthous stomatitis. The stimulation of tissue regeneration and modulation of inflammatory responses through micro needling may contribute to symptomatic relief and improved healing outcomes in these conditions [3].

Overall, the adaptation of microneedling techniques in dentistry represents an innovative approach to enhancing oral health outcomes and addressing various dental and periodontal concerns. As research in this area continues to evolve, microneedling holds potential as a valuable adjunctive therapy in the comprehensive management of oral diseases and conditions, offering patients a minimally invasive and effective treatment option within the dental setting.

What is Microneedling?

Micro-needling, also known as collagen induction therapy, is a minimally invasive cosmetic procedure that involves the use of a device containing fine needles to create controlled micro-injuries on the skin's surface. These micro-injuries stimulate the body's natural wound healing processes, leading to increased collagen and elastin production, which are essential for maintaining skin elasticity and firmness. The procedure is primarily used in dermatology and aesthetics to improve skin texture, reduce the appearance of scars, wrinkles, and fine lines, and to enhance overall skin rejuvenation. In dentistry it is also known as micropuncture or micropenetration therapy, is a minimally invasive technique used to stimulate tissue regeneration and enhance wound healing in the oral cavity. While microneedling is more commonly associated with dermatology for skin rejuvenation, its application in dentistry involves using fine needles to create controlled micro-injuries in the gums or oral mucosa [4].

History

The first recorded use and the first proven use of acupuncture is from a book called The Yellow Emperor‘s Classic of Internal Medicine, dating from 100 BC. The original purpose of acupuncture was to open channels. The first recorded use of a micro-needling procedure was in 1905 by German dermatologist Ernst Kromayer. He used various-sized dental burs powered by motor-driven flexible cord equipment to treat scars, birthmarks, and hyperpigmentation [5].

Micro-needling was first used in 1995 by Dr. Desmond Fernandes in Philadelphia to treat wrinkles and scars with hypodermic needles. Around the same time, Dr. Fernandes developed a small needle stamp to induce collagen production. Its original conception was given in 1995, when Orentreich and Orentreich [6] developed the concept of -subincision, or using hypodermic needles to induce wound healing in depressed cutaneous scars then independently in 1997 by a plastic surgeon Camirand who used tattoo guns without ink to take off tension from postsurgical scars. Micro-needling technique was given further shape by a German inventor Liebl in 2000 and a plastic surgeon Fernandes in 2006 who self-designed a drum-shaped device with multiple fine protruding needles and used it for percutaneous collagen induction.

Types of microneedles

1. Based on the method of manufacture, Microneedling can be classified based into following:

1) Structure in which are typically categorised as either in-plane or out-of-plane in literature.
2) Needle core we have solid and hollow.
3) Function.

a) Disposable manner.
b) Multi round responsive.
c) Temperature responsive.
d) Glucose responsive.
e) pH-responsive.
f) Swelling-shrinking.
g) Water-soluble.

4) Design, solid coated and designing. (Figure 1) The shaft of in-plane microneedles is produced parallel to the substrate surface. The ability

to precisely regulate the needle's length is an advantage of this setup. One drawback is that creating two-dimensional arrays is challenging. Conversely, out-of-plane microneedles are easily fabricated in array form and extend from the substrate. Rather, the manufacture of these kinds of needles becomes significantly challenging due to the length and high aspectratios. The solidity or hollowness of the microneedles is another important distinction to make. Active liquid transfer is made possible by hollow needles containing a needle bore, or lumen, inside the microneedle.[7] Microneedling can be classified based into following

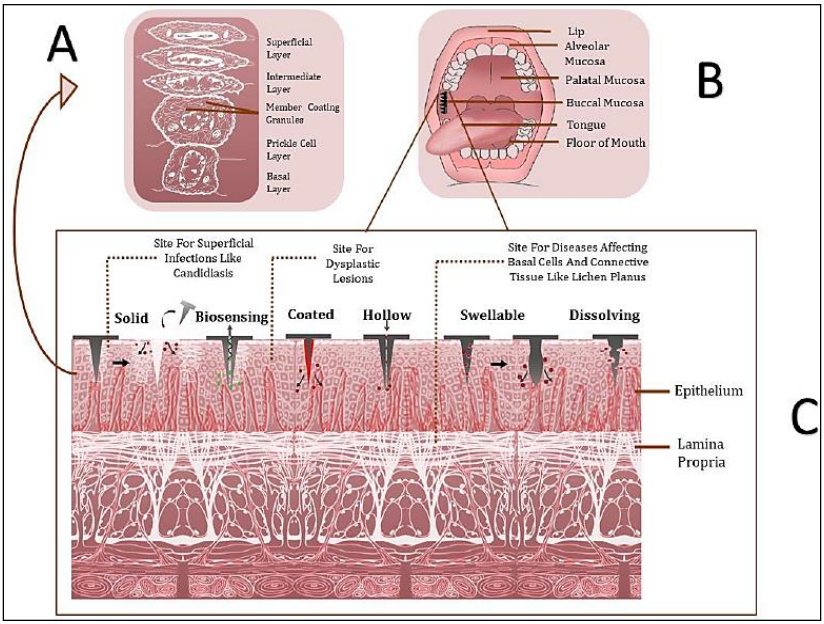

Fig 1: Structure of oral mucosa and the types of microneedles. A. Structure of oral epithelium; B. Needle based patch on buccal mucosa; C. Types of microneedles **(Image credits:** Batra P *et al*. Microneedles and Nanopatches-Based Delivery Devices in Dentistry. Discoveries (Craiova). 2020 Sep 30;8(3):e116.

Principle and mechanism of action

Micro-needling involves the production of transient epidermal and dermal pores ranging from 25 to 3,000µm in depth with the fundamental goal of upregulating normal skin synthesis mechanisms and not inducing prolonged inflammation or trauma that may lead to fibrosis. Microneedles cause micro punctures which produce a controlled skin injury without actually damaging the epidermis. These micro-injuries set up a wound healing cascade with the

release of various growth factors such as platelet-derived growth factor (PGF), transforming growth factor alpha and beta (TGF-α and TGF-β), connective tissue activating protein, connective tissue growth factor, and fibroblast growth factor (FGF) and leads to minimal superficial bleeding [8].

The old hardened scar strands are allowed to break down and re-vascularize but the help of a needle. Neovascularization and neo-collagenesis are initiated by migration and proliferation of fibroblasts and laying down of intercellular matrix. A fibronectin matrix forms after 5 days of injury that determines the deposition of collagen resulting in skin tightening persisting for 5-7 years in the form of collagen III. The depth of neo-collagenesis is 5-600 μm with a 1.5 mm length needle. Collagen fibre bundles appear to have a normal lattice pattern rather than parallel bundles as in scar tissue [9].

There are two major theories explaining how needles induce tissue remodelling and proliferation.

1) Fractionated Mechanical Microinjury.
2) Demarcation Current.

1. Fractionated mechanical micro-injury

The first explanation involves as fractionated mechanical micro injury. Needling forms tightly packed, minute perforations. The resultant near-confluent dermal and superficial capillary disruption provides a strong stimulus for growth factor release and fibroblast infiltration. Microchannels heal following the three classic phases of wound healing: inflammatory, proliferation, and remodelling. The inflammatory phase begins when disruption of the stratum corneum, endothelial lining, and sub endothelial matrix recruits' platelets and neutrophils to the site of injury. Needling expose. Thrombin and collagen fragments, which attract and activate platelets locally within minutes. The platelets, in turn, form a plug, initiate clotting, and release a myriad of growth factors and cytokines [10]. These signalling molecules control clot formation, increase vascular permeability, and attract leukocytes and fibroblasts to the site of injury. In the first 48 hours, neutrophils predominate; however, it is the subsequent influx of macrophages that is critical not only for phagocytosis but also to perpetuate signalling cascades that induce cell migration and division-without which healing fails to occur This also marks a transition to the proliferative phase of wound healing, during which growth factors derived from macrophages i.e., PDGF [platelet-derived growth factor], TGF-α [transforming growth factor] and TGF-β, interleukin-1, Tumor necrosis factor, FGF [fibroblast growth factor] recruit fibroblasts and alter extracellular matrix synthesis. Fibroblasts migrating along a

fibronectin scaffold allow for fibroplasia and granulation of the wound. Typically, in the healing of larger wounds, keratinocytes change their phenotype and migrate to repair tears in the basement membrane. Desmosomes dissolve and actin forms to knit keratinocytes together. The small injuries created by microneedles disrupt the basement membrane and expose keratinocytes to dermal collagen, thereby stimulating multiplication. However, these defects are close rapidly within hours-so the proliferating keratinocytes serve more to thicken the epidermis rather than close the needling channels [11].

At the same time, relative tissue hypoxia is another cue that triggers cytokine release, in response to which fibroblasts produce PDGF, FGF, and EGF. There is an upregulation of procollagen mRNA, which later converts to collagen III in the presence of oxygen supplied by new vessels. VEGF and other growth factors are key in the angiogenesis process. The remodelling begins in days with the formation of the fibronectin matrix but takes months to years for full execution. Collagen type I replaces III, which replaces the early granulation tissue laid down in the upper dermis. This procedure extrapolates the body's physiology of wound healing and the new collagen deposition results in skin tightening since the overlying epidermis is not ablated [12]. (Figure 2).

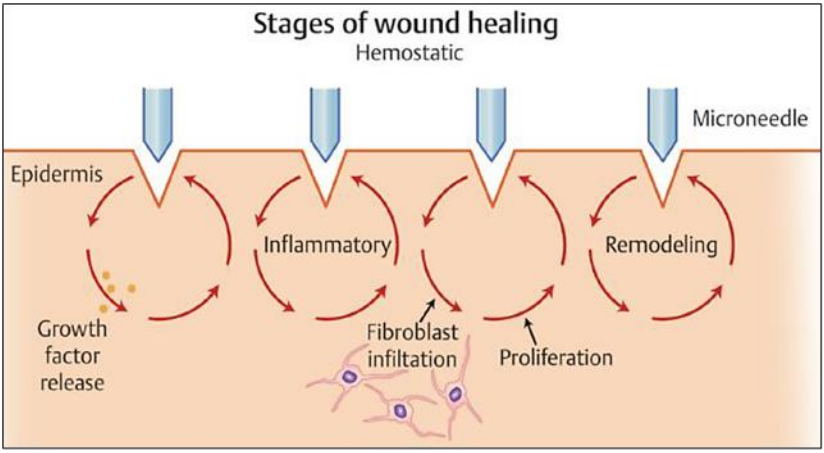

Fig 2: Fractionated mechanical microinjury
(Image Credits: Plastic Surgery key https://plasticsurgerykey.com microneedling mechanism and esthetical consideration 22 March 2020)

Demarcation current: Another hypothesis that explain how micro-needling works. The resting electrical membrane potential of cells is approximately -70 mV, and when needles come near the membrane, the inner

electrical potential increases quickly to -100 mV. This trigger increased cell activity and the release of various proteins, potassium, and growth factors from the cells into the exterior leading to the migration of fibroblasts to the site of injury, and hence, collagen induction. Thus, the needles do not create a wound in a real sense, but rather body cells are fooled into believing that the injury has occurred. The expression of matrix metalloproteinases induced. (Figure 3).

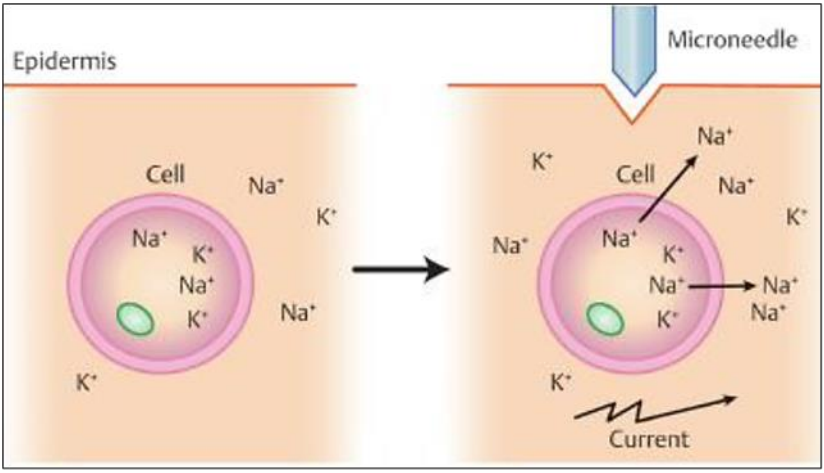

Fig 3: Demarcation Current
(Image Credits: Plastic Surgery key https://plasticsurgerykey.com micro needling mechanism and esthetical consideration 22 March 2020

Histologic changes after micro needling

Several studies have used histologic specimens and obtained at 3 and/or 6 months after therapy to document micro needling-induced epidermal and dermal changes. Aust *et al.* [14] showed those 4 monthly micro needling sessions triggered up to a 400% increase in collagen and elastin deposition within 6 months. This neo-collagenesis occurred in a normal basket weave configuration at a depth of up to 0.6mm with 1.5mm length needles. At one year, the investigators also noted a thickened stratum spinosum and normal rete ridge architecture compared to the flattening pattern seen over a cicatrix [15]. The increase in tissue elasticity lags behind the rise in collagen, where solar elastic fibres seen at baseline are cleared (drop in elastin content at 3 months) and trauma induces the production of new precursor elements that are later processed into more normal appearing elastic fibres (rise in elastin content at 6 months).

Potential use of microneedling in periodontology

Microneedling, a technique utilizing fine needles to create controlled micro-injuries in the skin, shows promise for various applications within dentistry, particularly in the field of periodontology (the study and treatment of gum diseases). Here are some potential uses of microneedling in dentistry, specifically in periodontology [16]:

1) **Gingival tissue regeneration:** Microneedling can be used to stimulate the regeneration of gum tissue. By creating micro-injuries in the gums, the body's natural healing response is triggered, leading to increased collagen production and enhanced tissue regeneration. This can be particularly beneficial for patients with receding gums or gingival defects.
2) **Enhanced delivery of therapeutic agents:** Microneedling can improve the penetration and absorption of topical medications or therapeutic agents into the gums. By creating micro-channels, microneedling allows for better delivery of antimicrobial agents, growth factors, or other substances directly into the periodontal tissues, potentially enhancing treatment outcomes [1].
3) **Reduction of periodontal pocket depth:** Microneedling may help reduce pocket depths in patients with periodontal disease. By promoting tissue regeneration and tightening of the gum tissue, microneedling could contribute to the reduction of periodontal pockets, leading to improved periodontal health.
4) **Management of oral mucosal disorders:** Microneedling can also be explored for managing oral mucosal disorders, such as lichen planus or leukoplakia. The controlled micro-injuries induced by microneedling may stimulate healing and regeneration of the oral mucosa, aiding in the treatment of certain oral lesions [17].
5) **Adjunct to Scaling and Root Planing (SRP):** Microneedling can potentially serve as an adjunct to traditional periodontal therapies like scaling and root planing (SRP). By preparing the periodontal tissues and enhancing their receptivity to treatment, microneedling may improve the overall effectiveness of SRP in controlling periodontal disease.
6) **Stimulation of collagen production:** Microneedling triggers the body's natural collagen production, which is essential for maintaining healthy periodontal tissues. Increased collagen synthesis can lead to improved tissue strength and resilience, potentially supporting long-term periodontal stability [18].

7) **Minimally invasive alternative:** Microneedling offers a minimally invasive alternative to surgical periodontal procedures. Patients may benefit from reduced discomfort, shorter recovery times, and fewer complications compared to traditional surgical interventions.

Research in these areas is ongoing, and further studies are needed to fully explore the potential benefits and optimal applications of microneedling in periodontology. However, early findings suggest that microneedling holds considerable promise as a valuable tool in the management and treatment of various periodontal conditions.

In addition to the points mentioned earlier, microneedling in dentistry, particularly in periodontology, holds potential for addressing and modifying gingival phenotype, which refers to the natural appearance and characteristics of the gingival tissue. Here's how microneedling could impact gingival phenotype:

8) **Modification of gingival contour and appearance:** Microneedling can be utilized to modify the contour and appearance of the gingiva. By stimulating collagen production and tissue regeneration, microneedling may help reshape uneven or asymmetrical gingival margins, resulting in a more esthetically pleasing gingival contour.[9] To the best of our knowledge MyM dermapen is used these days for microneedling to enhance gingival phenotype. (Figure 4)

Fig 4: My M Dermapen with 12 Pin Needle Cartridge Tip

9) **Treatment of gingival recession:** Microneedling shows promise in the treatment of gingival recession, a common condition where the gum tissue pulls away from the tooth, exposing the root surface. By promoting tissue regeneration and collagen synthesis, microneedling may encourage the growth of new gingival tissue to cover exposed roots, thus improving the appearance and health of the gums.

10) **Enhancement of attached gingiva:** Microneedling could potentially enhance the width and quality of attached gingiva, the firm, tightly bound gum tissue that surrounds the teeth. By stimulating collagen formation and tissue thickening, microneedling may contribute to the development of a broader zone of attached gingiva, which is essential for maintaining periodontal health and stability.
11) **Gingival pigmentation management:** Microneedling might be explored as a method for managing excessive gingival pigmentation (dark gums). By promoting controlled injury and subsequent regeneration, microneedling could facilitate the remodeling of pigmented gingival tissues, resulting in a more uniform and lighter gingival coloration [11].
12) **Customized treatment for different gingival phenotypes:** Microneedling can potentially be customized based on individual gingival phenotypes. Different microneedling techniques and depths of needle penetration can be tailored to address specific gingival characteristics and achieve optimal therapeutic outcomes for each patient.
13) **Adjunct to esthetic periodontal procedures:** Microneedling may serve as an adjunct to various esthetic periodontal procedures, such as gingival grafting or crown lengthening. By preparing the gingival tissues and improving their receptivity to subsequent interventions, microneedling could enhance the overall success and esthetic results of these procedures.

In summary, microneedling offers a versatile approach to modifying and improving gingival phenotype in the context of periodontology. Through its ability to stimulate tissue regeneration, collagen production, and controlled wound healing, microneedling holds promise for addressing gingival concerns and optimizing the esthetic and functional outcomes of periodontal treatments. Further research and clinical studies are warranted to establish the specific protocols and effectiveness of microneedling in managing gingival phenotype across different patient populations.

Conclusion

In conclusion, microneedling shows promise as a beneficial treatment modality in dentistry. Through its ability to stimulate collagen production, improve wound healing, and potentially enhance the outcomes of procedures like bone grafting, gingival augmentation, implant placement, micro needling holds potential for improving patient outcomes and satisfaction in dental

practice. However, further research, particularly well-controlled clinical trials, is necessary to fully understand its efficacy, safety profile, and optimal protocols for various dental applications. With continued investigation and refinement, micro needling could become a valuable adjunctive therapy in the dental field, offering new avenues for enhanced patient care and treatment success.

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Chapter - 5
Precision Medicine in Periodontal Therapy: A Comprehensive Review of Local Drug Delivery Advancements

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Chapter - 5

Precision Medicine in Periodontal Therapy: A Comprehensive Review of Local Drug Delivery Advancements

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Abstract

Periodontitis is a destructive inflammatory disease of the periodontium induced by specific microorganisms and requires more specific treatment. The traditional mechanical therapy alone is not sufficient for the treatment of moderate to severe periodontitis because of in accessibility in the deep periodontal pocket and depth of penetration of microorganisms into the periodontal connective tissues. Thus, to overcome the limitations of mechanical therapy, local drug delivery into the periodontal pocket is recommended. The local drug delivery of chemotherapeutic agents to the periodontal lesion site has the advantage of loading a higher concentration of drug at the target site minimizing the adverse effect of the drug on the other systems of the body. The local drug delivery system having controlled release should be considered as an adjunctive to mechanical debridement for the treatment of periodontal diseases. This book chapter delves into the latest advancements in local drug delivery for periodontal therapy. It highlights innovative approaches and technologies aimed at enhancing the efficacy and precision of treatment, offering a comprehensive overview of this rapidly evolving field.

Keywords: Local drug, periodontitis, infection, mechanical therapy, concentration

Introduction

Periodontitis is defined as an inflammatory disease of supporting tissues of teeth initiated by specific microorganisms or groups of specific microorganisms, resulting in progressive destruction of the periodontium with periodontal pocket formation, gingival recession, or combination of both [1]. Periodontitis is multi factorial disease of periodontium initiated by a periodontal pathogenic microorganism and modified by the factors such as

developmental deformities of the tooth, systemic conditions affecting oral tissues, environmental factors, socioeconomic factors, and stress.

The nature of the periodontal disease depends on the interaction among the microorganisms, the oral environment, and the host's defense mechanisms to the bacterial assault, mainly composed of gram-negative anaerobic bacteria [2]. This pathogenic microbiota occurs due to the accumulation of subgingival plaque. The current concept of treating periodontal diseases is based on eliminating oral biofilms. A fundamental objective of periodontal therapy is to reduce or possibly eliminate the patho genicity of the periodontal pathogenic microorganisms in the subgingival periodontal area. Theoretically, there are two approaches in periodontal therapy:

To reduce or eliminate the total plaque microflora by mechno therapy, such as scaling and root planing, to reduce or eliminate the specific pathogens in the subgingival plaque using antimicrobial agents as an adjunct to mechano therapy. The traditional treatment of periodontitis involves oral prophylaxis, which includes patient motivation, education regarding periodontal diseases, oral hygiene maintenance methods, professional mechanical debridement like scaling and root planing, air abrasive polishing system. Mechanical debridement aimed at removing the suprgingival and subgingival microflora and creating hard, clean, smooth and compatible root surfaces. But in several conditions, the complex anatomy of the root and the location of the periodontal lesion may affect the ideal outcome of treatment and prevent the adequate reduction of the microbial load.

The effectiveness of mechanical therapy is limited due to the lack of accessibility in deep periodontal pockets [3]. Putative pathogens associated with periodontal diseases are susceptible to a variety of antiseptics and antimicrobials [4]. Antimicrobials have been used as an adjunct with mechanical debridement in the management of periodontal infections. For the successful outcome of periodontal therapy, antimicrobial agents must reach beyond the depth of the periodontal pocket and produce gingival crevicular fluid concentration more than the Minimum Inhibitory Concentration (MIC) of the suspected periodontal microbes. Antimicrobial agents have been administered both systemically and locally. Systemic administration is usually indicated as an adjunct to scaling and root planning in order to prevent the recolonization of pathogenic microorganisms.

It is administered for a period of 7-14 days. But it requires a higher concentration to be administered every few hours in order to stabilize the effective dose level. It may lead to adverse effects like hypersensitivity

reaction, GIT disturbances and bacterial resistance. These adverse effects would markedly be reduced if the antimicrobial agents are delivered locally into the periodontal pockets. Therefore, local drug delivery used as an adjunct to scaling and root planing helps in the control of growth of pathogenic bacteria.

The principal requirement for the effectiveness of local drug delivery is that the agent should reach at the base of the periodontal pocket and the concentration should be maintained at the site by means like a reservoir for an adequate time for the antimicrobial effect to occur [5]. It was in the year 1979 when Dr. Max Goodson *et al*. [6] first proposed the concept of controlled release drug delivery in the treatment of periodontitis.

Classification

Various classification systems of local drug delivery systems were evolved.

A. Based on the application rams and slots (1996) [7]

Personally applied (Patient self-care application)

- Non sustained subgingival drug delivery.
- Home oral irrigation.
- Home oral irrigation jet tips.
- Traditional jet tips.
- Oral irrigation (water pick).
- Soft cone rubber tips (Pickpocket).
- Sustained subgingival drug delivery.

Professionally applied in dental office

- Non sustained subgingival drug delivery.
- Professional pocket irrigation.
- Sustained subgingival drug delivery.
- Controlled release devices.
- Hollow fibers.
- Dialysis tubing.
- Strips.
- Films.

B. Based on the duration of medicament release Greenstein and Tonetti (2000) [7]

Sustained release devices

- These drugs provide drug delivery for less than 24hrs.
- Require multiple applications.
- Follow first-order drug kinetics.

Controlled release devices

- Drug release is more than 24hrs.
- Administered only once.
- Follow zero-order drug kinetics.

C. Depending on degradability [8]

- Non-degradable devices (First generation).
- Degradable devices (Second generation).

D. Langer and Peppas (1989) [9]: Classified controlled drug release polymeric systems based on their mechanism of action

- Diffusion Controlled System.
- Matrices.
- Reservoirs.

Chemically controlled systems

- Erodible systems.
- Pendant chain systems.

Solvent activated systems

- Osmotic systems.
- Swelling controlled systems.

Release induced by external forces

E. Kornman [10] (1993) has classified the controlled release local drug delivery system as:

- Reservoirs without a rate controlling system like hollow fibers, gels and dialysis tubing.
- Reservoirs with a rate controlling system like erodible polymeric matrices, micro-porous polymer membrane, monolithic matrices and coated drug particles.

F. Depending on the origin

- Allopathic or chemical local drug delivery.
- Herbal or ayurvedic local drug delivery.

G. According to WHO guidelines, herbal medicines can be categorized into four categories [11]

Based on their evolution, origin, andforms of current usage as under:-

Category 1: These indigenous herbal medicines are used by local communities or region and are very well-known by the local population through ages in context to composition, treatment, and dosage.

Category 2: This group consists of herbal medicines in systems and is well-documented and based on long-time usage on theories and concepts that are duly accepted by the respective countries. Example-Ayurveda Siddha and Unani.

Category 3: This consists of Modified herbal medicines which have been modified in relation to their shape, dose, administration mode, and composition. These medicines have to meet the national regulatory requirements in terms of their safety and efficacy.

Category 4: Imported products with a herbal medicine base include all the imported herbal medicines (raw materials and products). The national authority of the importing country should have safety and efficacy data.

H. Based on the types of local drug delivery system [11]

- Fibers.
- Films.
- Strips.
- Gels.
- Vesicular liposomal systems.
- Microparticle systems.
- Nanoparticle systems.

Principle

Oral administration of antibiotics has various adverse effects like inadequate drug concentration in they periodontal pocket, problems related to patient compliance, rapid fall in concentration to sub therapeutic level demanding repeated drug placement and development of bacterial resistance. Periodontal pocket bathed by GCF (Gingival Crevicular Fluid) offers an

accessible natural reservoir for placement of LDD device. Local drug delivery provides a leeching medium for the release of a drug from solid dosage form and for its distribution throughout the pocket.

The objective of using an intra-pocket device is to obtain and maintain therapeutic levels of drug for required period of time that causes inhibition or killing of microorganism without any harm to the tissues. Controlled delivery systems are released slowly for sustained drug action and prolonged drug availability. Thus, periodontal pocket is the most appropriate site for placement of LDD and is considered the best modality [12].

Indications [8]

- Patients with recurrent/refractory periodontitis.
- Patients under supportive periodontal therapy/maintenance therapy.
- As an adjunct to SRP.
- Isolated periodontal pocket of 5 mm after successful phase I therapy.
- Medically compromised patients who either cannot undergo surgical therapy or who refuse to surgical periodontal therapy.
- During regenerative periodontal surgical procedures.
- Since sustained release devices are less invasive and less time consuming when compared to surgical treatment, LDD can be advantageous in patients with moderate periodontitis to restrict the extent of degree of surgical intervention required in the future.

Contraindications [8]

- Patients with known hypersensitivity to the antimicrobials used.
- Patients susceptible to bacterial endocarditis who are contraindicated for subgingival irrigation devices to avoid the risk of bacteraemia.
- Delivery of antimicrobials using ultrasonic devices is contraindicated in asthmatics, infective conditions such as AIDS, tuberculosis and those with cardiac pacemakers.
- In pregnant and lactating mothers where use of the particular antimicrobial are to be avoided.

Ideal requisite of local drug delivery system [6]

- Local drug delivery system should deliver drug to the connective tissue apically and laterally of the periodontal pocket.
- Local drug delivery system should deliver drug at a microbiologically effective concentration.

- Local drug delivery system should sustain the concentration of drugs in the pocket for sufficient time and concentration to be clinically effective.
- It should be easy to deliver into the periodontal pocket.
- It should retain at the proximity of periodontal pocket after placement.
- It should be biodegradable.
- It should not develop bacterial resistance.
- It should be safe without any adverse effects.
- It should not affect the commensal microflora of periodontal pocket.

Advantages [10]

- When compared to systemic drug regimen, LDD attains 100- fold higher concentrations in subgingival site.
- Employs antimicrobial agents not suitable for systemic administration, such as various broad-spectrum antiseptic solutions.
- Better compliance.
- Decreases the risk of development of drug resistant microbial populations at non-oral body sites.
- Less invasive when compared to surgical intervention.

Disadvantages [10]

- Difficulty in placement (deeper periodontal pockets/furcation areas).
- Time consuming and labour intensive.
- Use of LDD into periodontal pocket does not significantly affect the periodontal pathogens harbouring in the adjacent oral structures like tonsils, tongue etc. Hence, there is increased risk of recurrence of the disease.
- Relatively expensive.

Devices used as local drug delivery

Fibers

These are threadlike, reservoir-type drug delivery systems that can be inserted and positioned in place by cyanoacrylate adhesive, thereby providing sustained release of tetracycline within the pocket. Goodson and co-workers developed tetracycline hydrochloride fibers [13]. Single application of these

fibres resulted in significant reduction in spirochetes population. However, since the fibres were hollow it resulted in rapid draining of the drug. Therefore, to overthrow this limitation, matrix type of fibres was introduced to obtain tetracycline loaded ethyl vinyl acetate fibres, commercially available as Actisite. However, these were non-bioabsorbable, so once exhausted they required replacement with new ones. Other polymers used so far contain nylon, chitosan, polycaprolactone, collagen and alginate. Use of tetracycline fibres had an advantage of improving clinical parameters but were associated with a number of disadvantages like placement was time consuming, associated patient discomfort, was associated with redness and dislodgement of the fibers. Hence its use proved to be problematic for both clinician and the patient.

Films

Films are one of the most commonly used local drug delivery devices available and are prepared either by direct milling or solvent casting. They consist of drugs dispersed throughout the polymer and the ones made of non-degradable/water insoluble polymers are released by simple diffusion only while the films made of biodegradable/soluble polymers are released either by diffusion/matrix dissolution or erosion. Films can be sectioned into desired dimension and positioned into the periodontal pocket. Use of films has several advantages like: Easy insertion, customizability and less discomfort to the patient [12]. Films with sufficient tackiness and a width of <400 mm cannot be easily dislodged during routine oral hygiene procedures performed by the patient. Ethyl cellulose films integrating various drugs like metronidazole, tetracycline, chlorhexidine diacetate and minocycline have been developed.

Gels

Gels are semisolid system that are used for targeted delivery of antibiotics. They possess various advantages like: easy preparation and administration, faster drug release rate, biocompatible and bio-adhesive nature, therefore they stick to the periodontal pocket, also they have a minimum risk of irritation/allergic host reactions on the site of application since they can be quickly eliminated though catabolic pathway. Gels can be either oleogels or hydrogels. Hydrogels comprise of 3 dimensional cross-linked structures of ionic interaction and hydrogen bonding and can be either macroporous, microporous or nonporous with a pore size of 0.1-1 mm and 100-1000 A˚ respectively with a potential to absorb water (10-20 times than their molecular weight) and therefore causing them to swell-up [13].

Strips and Compacts

A strip is a thin and elongated matrix band comprising of an elastic polymer with drugs dispersed throughout the polymer and it occupies a wide range of interproximal area. Strips are fabricated either by pressure melt method or solvent casting. Acrylic strips made of single or a combination of drugs has been used by researchers in the past which bought remarkable improvement in clinical parameters by effective elimination of microbes from periodontal pocket. However, strips have their own limitation. Since the strips are non-biodegradable, they require removal after therapy, which may interfere with regeneration of the tissues at the placement site.

Vesicular systems

Liposomes are similar to biomembranes in structure and behaviour; therefore, they are exclusively used in periodontal diseases. Liposomes are microscopic lipid-based vesicles and can be either uni/multi lamellar. They are manufactured using long chain fatty acids, non-toxic surfactants, cholesterol, membrane proteins, sphingolipids and glucolipids Liposomes are formed by self-assembling phospholipids in aqueous medium and can vary in size, charge, composition, lamellarity, lipids and method of preparation. Size of vesicles range between 0.1-0.5 mm for multilamellar, 0.02-0.05 mm for small unilamellar and >0.06 mm for large unilamellar respectively. They have various advantages like: biocompatibility, biodegradability, nonimmunogenic, non-toxic, good stability and ability to protect the drug from external environment. Nonetheless they have various disadvantages: leakage of the encapsulated drugs, high production cost and shorter half-life.

Microparticle system

Microspheres are solid spherical structures containing drugs dispersed in polymeric matrix. They offer controlled and sustained drug release at the site of insertion. They can be either biodegradable or non-biodegradable and can be either obtained from natural or synthetic source. They can be used to dental paste chip, or can be injected directly into the periodontal pocket. Microparticles have various advantages which include improved patient compliance, decreased intensity and frequency of adverse effects, guarding of unstable drug before and after administration, sustained therapeutic effect, and enhanced bioavailability and controlled drug release.

Nanoparticle system

Drug release of micro particle-based hydrogels used in dentistry is affected by their structure. Nanoparticles are more advantageous than

emulsion-based delivery systems microparticles and microspheres. They have a size range from 10-1000 nm. The drug is attached, encapsulated, entrapped or dissolved to a nanoparticle matrix. They are very dispersible in aqueous medium, offer controlled release rate and enhanced stability.

Since they have a small size, they can reach sites that cannot be reached by other devices. An equal drug distribution for extended period of time is achieved with nanoparticles with an additional advantage of decreasing the dosage frequency.

Nano fibers

Nanofiber technology is a new and developing field. Polymeric fibres have a size range of nanometre or submicron order and are referred to as nanofibers [13]. They provide numerous remarkable physical properties like better strength and a larger surface area to volume ratio. Preparation of nanofibers is carried out by various methods and electro spinning is the most accepted method for their manufacture.

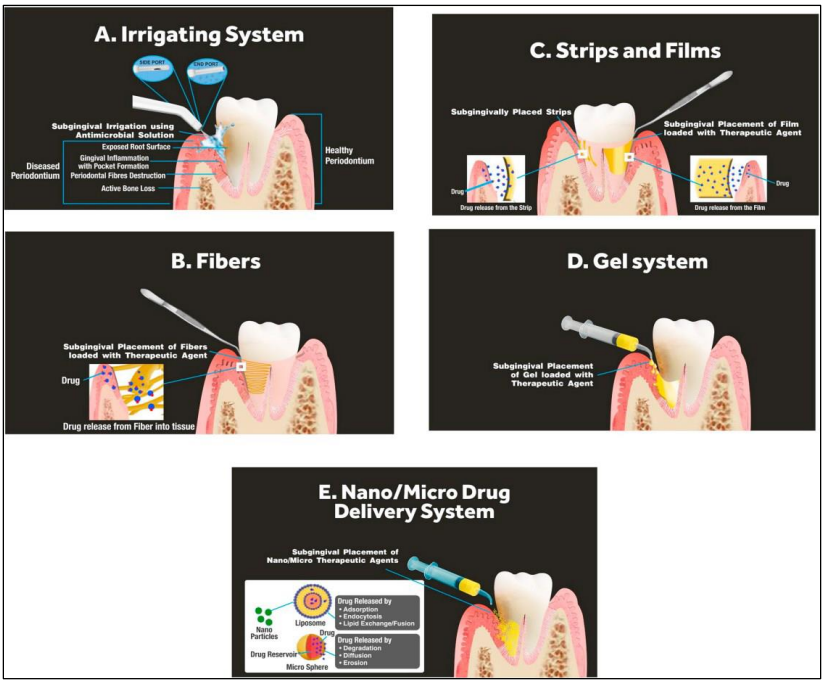

Fig 1: Local drug delivery in periodontics. A. Oral irrigating system, B. Fibers, C. Strips and films, D. Gels, E. Nano/Micro delivery system

Various chemical agents used as local drug delivery

Tetracycline: These are broad-spectrum antibiotics that are more effective against gram-positive bacteria. Tetracycline is a bacteriostatic antibiotic that interferes with bacterial protein synthesis and inhibits tissue collagenase activity. Tetracycline achieves 2 to 10 times more concentrated in gingival crevicular fluid than the concentration in the serum. Tetracycline is available as biodegradable and non-biodegradable form for the local drug delivery system.

Actisite: Actisite was the first commercial lised, controlled-release antimicrobial local drug delivery product introduced in 1994. It is 23 cm long with 0.55 mm diameter, delivering 12.7 mg of tetracycline hydrochloride. The fibre is non-biodegradable and has to be retrieved after 10 days which is the disadvantage of this system. Actisite achieves a concentration of 1590 µg/ml of gingival crevicular fluid in the periodontal pocket however, 250 mg of oral tetracycline achieves a concentration of 1 µg/ml in the gingival crevicular fluid. The concentration level remained at a mean of 1300 µg/ml for 7 days in local drug delivery of Actisite [9].

Periodontal plus AB: It biodegrades within 7 days, so there is no need for a second appointment. Collagen fibril-based formulation contains tetracycline hydrochloride (2 mg of tetracycline) in which 25 mg are collagen fibrils that can be directly applied for all levels of periodontal infections. The application of tetracycline fibers as an adjunct to scaling and root planing and found it to be more effective in reducing gingival and periodontal inflammation (P=>0.05) [10].

Minocycline: It is a broad-spectrum antibiotic that is a derivative of tetracycline. It exhibits bacteriostatic action. It has greater substantivity and lipid solubility than tetracycline. It is available in various forms such as film, microspheres, ointment, and gel.

Film: Ethylcellulose film with 30% minocycline is used as a sustained-release device drug delivery system.

Microsphere: FDA has approved sustained-release minocycline microspheres as a local drug delivery system. It is commercially available by trade name as ARESTIN. ARESTIN is a 2% minocycline encapsulated into bio-resorbable microspheres with 20-60µm diameter in a gel carrier and it has a resorption time of 21 days.

Ointment: The 2% minocycline hydrochloride in a matrix of hydroxyethyl-cellulose, amino alkyl-methacrylate, triacetine & glycerine. The

concentration of minocycline in the periodontal pocket is about 1300 µg/ml of gingival crevicular fluid, 1 h after single topical application of 0.05 ml ointment and is reduced to 90µg/ml after 7 h [11]. It is available by the trade name DENTOMYCIN and PERIOCLINE in European Union and JAPAN, respectively.

Doxycycline: Doxycycline is a bacteriostatic agent. Its effective against the matrix metalloproteinase (MMP's) and has the ability to down-regulate matrix metalloproteinase (MMP's) [7]. MMP's are the periodontal biomarkers causing the destruction of periodontal connective tissue. ATRIDOX (42.5 mg Doxycycline) is the only subgingival controlled-release local drug delivery system, a commercially available product of doxycycline composed of a two syringe mixing system. It is the only FDA-approved 10% Doxycycline gel accepted by ADA. Doxycycline levels in GCF peaked to 1,500-2000 µg/ml of GCF in 2 h following the delivery of ATRIDOX. Local levels of Doxycycline have been found to remain well above the MIC for periodontal pathogens (6.0 µg/ml) for the 7 days. Within 28 days the 95% of the polymer of ATRIDOX is bio absorbed or expelled from the periodontal pocket [11].

Chlorhexidine: It is an anti-fungal and anti-bacterial agent, available as mouth rinses, gels, varnishes, and chips. CHX acts by binding to anionic acid groups on salivary glycoproteins, thus reducing pellicle formation and colonization of microorganisms in the plaque. Chlorhexidine has been shown to be an effective agent with higher substantivity. It has greater affinity for hydroxyapetite and acidic salivary protein. Due to increased cellular membrane permeability and ability to induce the coagulation of intracellular cytoplasm macro-molecule it is effective as an antibacterial agent.

Periochip: It is a baby nail-sized chip composed of biodegradable hydrolyzed gelatin matrix, cross-linked with glutaraldehyde containing glycerine & water. The dimension of periochip is 4 × 5 × 0.35 mm with an available concentration of chlorhexidine 2.5 mg. *In-vitro* study, it is evaluated that periochip releases chlorhexidine in a biphasic manner, initially releasing approximately 40% of the chlorhexidine within the first 24 h and then releasing the remaining chlorhexidine in linear fashion for 7-10 days 30. One border of periochip is rounded which is directed apically while inserting into the periodontal pocket to avoid mechanical trauma to the sulcular and junctional epithelium.

Periocol-CG: Periocol CG is also a chlorhexidine chip with a dimension of 4 × 5 × 0.25 to 0.32 mm prepared by incorporating 2.5 mg chlorhexidine from a 20% chlorhexidine solution in a collagen membrane. The weight of Periocol CG is 10 mg.

Chlo-site: It is an agent containing 1.5% chlorhexidine in xanthan type. Xanthan gel is a saccharide polymer, which constitutes of a three-dimensional mesh mechanism, which is biocompatible with chlorhexidine.

Metronidazole: It is a nitroimidazole compound. It is bacteriocidal to anaerobic organism. It acts by disrupting bacterial DNA synthesis. After delivery of Elyzol (25% Metronidazole), concentrations of above 100 μg/ml of drug in GCF were measurable in the periodontal pocket for at least 8 h, and concentrations above 1 μ/ml were found after 36 h. It is delivered in viscous consistency to the pocket, where it is liquidized by the body heat and then hardens again, and forming crystals in contact with GCF or saliva [11].

Satranidazole (SZ): It is another antibiotic that belongs to the 5-nitroimidazole group. SZ, (1-ethylsulphonyl-3-[1-methyl-5-nitro-2-imidazolyl]-2-imidazolidinone) is a novel nitroimidazole which differs from other 5-nitroimidazoles such as metronidazole, ornidazole, and tinidazole, in that 2 °C of the imidazole ring is connected through nitrogen to a substituted imidazolidinone. Satranidazole gel, when used as an adjunct with scaling and root planing in the management of periodontitis, achieves significantly better clinical and microbiological results than mechanical periodontal treatment alone [13].

Herbal agents used as local drug delivery

Recently, usage of the herbal product has increased because of the relatively safe nature of herbal extracts; many herbal products and their components are being used for treating periodontitis in the form of local drugs delivery.

Neem: Neem leaf extract can help reduce bacterial load in dental plaque levels that cause the initiation and progression of periodontitis. It is evaluated that bioactive materials available in neem leads to the presence of gallotannins during the early stages of plaque formation that could effectively reduce the bacterial load in dental plaque and help removal of plaque from the tooth surface and oral cavity through the aggregate formation. Additionally, the effective inhibition of glucosyl transferase activity and the reduced bacterial adhesion to saliva-coated hydroxyl appetite suggest some potential anti-plaque activity [12].

Aloe vera: *Aloe vera* is the commonly used medicinal cactus plant that belongs to the Liliaceae family. More than 300 species of aloe plants exist in the world, but only two species have been studied for local drug delivery, which are Aloe barbadensis Miller and Aloe arborescence. *Aloe vera* is having anti-inflammatory, antibacterial, antioxidant, antiviral, and antifungal actions

as well as producing hypoglycemic effects. It is effective in reducing gingival bleeding, inflammation, and swelling. It is a powerful healing promoter and can be used following tooth extractions also [13].

Lemon grass: It is a popular medicinal plant. This plant is commonly used in teas, cosmetics, and folk medicine for its antiseptic, antiemetic, anti-rheumatic, analgesic, antispasmodic, and antipyretic properties.

Its chemical components like phenol and flavanoid substances were reported to show many *in-vitro* and *in-vivo* biological activities such as antioxidant, anti-inflammatory, and anti-mutagenic activities. At a concentration of 2% lemongrass, essential oil appears to be an effective local drug delivery agent as an adjunct to mechanical nonsurgical periodontal therapy [6].

Green tea: Green tea is an effective local drug delivery agent as it contains a number of bioactive chemicals such as flavonoids, including catechins and their derivatives. Green tea is rich in therapeutic effects such as antioxidant, anti-collagenase, anti-inflammatory, anti-caries, anti-fungal, antiviral and antibacterial effects.

Tea tree oil: Tea tree oil (TTO) is derived from the paperbark tea tree. Tea tree oil has a broad-spectrum antimicrobial, antifungal, antiviral, antioxidant, and anti-inflammatory effect. Elgendy EA suggested that TTO is effective as an adjunctive treatment of scaling and root planing on the clinical parameters.

Curcumin: Turmeric (Curcuma longa) is an Indian spice derived from the rhizomes, a perennial member of the Zingiberaceae family. Turmeric is rich in curcuminoids such as curcumin (diferuloylmethane), demethoxycurcumin, and bisdemethoxy curcumin as well as volatile oils (turmerone, atlantone, and zingiberene), sugars, proteins, and resins. Curcumin exhibits anti-inflammatory, antioxidant, anticarcinogenic, antiviral, and antimicrobial activities. Curcumin modulates the inflammatory response by down-regulating the activity of cyclooxygenase-2, lipoxygenase, and inducible nitric oxide synthase enzymes and inhibits the production of the inflammatory cytokines [12].

Oak: Oak is a species from the Fagacea family, which is grown in Western Iran. Oak is rich in hemostatic, anti-bacterial, anti-inflammatory, anti-nociceptive, and anti-oxidant effects and has been traditionally used for the treatment of gastric ulcers, superficial injuries, and local inflammation. Oak has been evaluated as a local drug delivery agent in periodontal diseases [10].

Coriander: Coriander Sativumis from the Umbelliferae family was used in Iranian folk medicine as a carminative and spasmolytic agent. It is effective local drug delivery agent as it has anti-inflammatory, analgesic, anti-bacterial and anti-oxidant activities. *C. sativum* extract has tannins also.

Babul: Babul has cyanogenic glycosides in addition to several enzymes such as oxidases, peroxidases, and pectinases that have shown to inhibit microbial growth. Its bark contains tannins (24-42%) which have analgesic, anti-inflammatory properties.

Bakul: One of the major pharmacologically active ingredients lupeol is present in bakul has anti-inflammatory and anti-microbial properties, which has attracted the researchers' attention towards Bakul for scientific studies [7].

Pomegranate: Pomegranate has active compounds containing polyphenolic flavonoids (e.g. Punicalagins and ellagic acid) are believed to prevent gingivitis through a number of mechanisms, including reduction of oxidative stress in the oral cavity, antioxidant activity, anti-inflammatory effects and anti-bacterial effects, so rinsing with pomegranate lowers the activity of alfa glucuronidase, an enzyme that breaks down sucrose while it increased the activities of ceruloplasmin, an antioxidant enzyme.

Recent developments in local drug delivery in periodontal treatment

Eradication of microorganisms from the periodontal pocket is the most important step in treating periodontitis. The limitations of mouth rinsing and irrigation have prompted research for the development of alternative delivery systems. The requirements for treating periodontal disease include a means for targeting an anti-infective agent to infection sites and sustaining its localized concentration at effective levels for a sufficient time while concurrently evoking minimal or no side effects. Various newer agents are being investigated in the field of local drug delivery to ensure maximum benefit.

1. Clarithromycin gel

A study has been conducted to investigate the adjunctive effects of subgingivally delivered 0.5 % clarithromycin as an adjunct to scaling and root planing for treating chronic periodontitis smoker subjects. It was observed that the adjunctive use of 0.5% clarithromycin as a controlled drug delivery system enhanced the clinical outcome. At the end of 6 months, the mean GI, PI, SBI, PPD, CAL for the clarithromycin group was significantly reduced. This product is still under investigation and yet to be patented [15].

2. Herbal products

Various herbal formulations like aloe vera, neem, tulsi, propolis, cocoa

husk, pomegranate, cranberry etc. are being used widely these days [16]. These products have shown promising results with no side effects and are economical as well.

3. Okade and co-workers developed a new sub gingival release delivery system (PT-01) containing Ofloxacin for sub gingival therapy. The PT-01 was found to be effective in the reduction of supra gingival plaque, reduction in the plaque index, reduction in bleeding on probing [14].
4. Fibroblast growth factor would be a very efficacious introduction in local drug delivery. To regenerate periodontal tissues, a sandwich membrane composed of a collagen sponge scaffold and gelatin microspheres containing basic fibroblast growth factor (bFGF) in a controlled-release system was developed. This sandwich membrane induced successful regeneration of the periodontal tissues in a short period of time (4 weeks) [16].
5. Colloidal drug carriers include micelles, emulsions, liposomes and nanoparticles (nanospheres and nanocapsules). It is noteworthy that only liposomes and nanoparticles have been largely exploited for drug delivery because the methods of preparation are generally simple and easy to scale-up**.** The aim of using colloidal carriers is generally, to increase the specificity towards cells or tissues, to improve the bioavailability of drugs by increasing their diffusion through biological membranes and/or to protect them against enzyme inactivation [13].

Nanoparticles, owing to their small size, penetrate regions that may be inaccessible to other delivery systems. These systems reduce the frequency of administration and further provide a uniform distribution of the active agent over an extended period of time. Three preliminary studies have been conducted to assess the efficacy of nanoparticles in periodontal drug delivery.

a) Antisense oligonucleotide-loaded chitosantripolyphosphate (TPP) nanoparticles and showed the sustained release of oligonucleotides which is suitable for the local therapeutic application in periodontal diseases.[13]
b) Preliminary *in vivo* study in dogs with induced periodontal defects using Triclosan-loaded polymeric (PLGA, PLA and cellulose acetate phthalate) nanoparticles and suggested that triclosan-loaded nanoparticles penetrate through the junctional epithelium.[15]
c) Bactericidal activity of the Harungana madagascariensis leaf extract

(HLE) on the oral bacterial strains largely implicated in dental caries and gingivitis infections.

HLE-loaded PLGA nanoparticles were prepared using interfacial polymer deposition following the solvent diffusion method. Incorporation of the HLE into a colloidal carrier improved its antibacterial performance and diminution of the bactericidal concentration was observed [14].

Conclusion

The local drug delivery devices are a useful adjunct to conventional surgical or non-surgical periodontal therapy but are no substitute for these measures. Controlled release delivery drug systems containing antibacterial, anti-inflammatory, anti-oxidant properties can be used effectively in the management of periodontitis. The local drug delivery provides a better improvement in periodontal conditions. Various chemical and herbal products are evaluated in local drug delivery systems with controlled release properties. It aims to minimize drug degradation and loss, prevent harmful adverse effects and increase drug bioavailability at the site of the lesion.

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Chapter - 6
Innovative uses of Platelet Concentrates for Tissue Regeneration

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Chapter - 6

Innovative uses of Platelet Concentrates for Tissue Regeneration

Dr. Kumari Pallavi and Dr. Neelam Das

Abstract

The appropriateness of biocompatible materials for regenerative medicine has been the subject of several investigations. Platelet-rich plasma, platelet-rich fibrin, and concentrated growth factor are some of the biological properties that give rise to the names of the platelet concentrates, which are obtained from centrifuged blood. In both soft and hard tissue engineering, platelet concentrates have drawn a lot of interest. In reality, growth factors, platelets, and fibrin matrix are only a few of the components of autologous platelet concentrates that play crucial roles in wound healing. Present research endeavours centre on innovative approaches to fulfil tissue repair prerequisites by enhancing the attributes of autologous platelet concentrates. Applications of platelet concentrates for tissue engineering are explored in the current study, which includes a summary of recent developments and innovative techniques. Furthermore, a number of these tactics are examined, including the benefits of lyophilized platelet concentrates and the mixing of platelet concentrates with biomaterials, stem cells, or medications.

Keywords: Platelet concentrates, platelet-rich fibrin, wound healing, tissue regeneration, growth factors

Introduction

Regenerative medicine and tissue engineering aim to restore both the structure and functions of damaged tissues. In recent years, novel and more efficient regenerative strategies have been devised. These emerging approaches employ specific biocompatible and bioactive composite or natural scaffolds, into which cells or bioactive molecules are incorporated to construct a dynamic environment for wound healing in damaged tissues. However, the development and optimization of low-cost and effective therapeutic methods remains the primary goal [1].

Several studies have focused on autologous platelet concentrate derivatives, which may delay complications and boost tissue regeneration. It has been >40 years since the first generation of autologous platelet concentrates, termed platelet-rich plasma (PRP), was produced [2]. Since then, other platelet concentrates have been established, including platelet-rich fibrin (PRF) and concentrated growth factor (CGF).

Tissue regeneration is a thought-provoking area in the field of dentistry. Several materials have been used in the past with varied success. Incorporation of biologically active molecules particularly growth factors have yielded promising results amongst which platelets had been proven to be a good source of these factors.

In addition to its role in regular hemostasis platelets have a wide array of applications which had evinced clinical interest of late in platelet derived products and lead to the evolution of platelet concentrates categorized as first-generation platelet concentrates such as platelet rich plasma (PRP) and second generation such as platelet rich fibrin (PRF).

Historical background

Regenerative potential of platelets was introduced in 1970's and its clinical applications began in the field of medicine in 1980's. From then it took a decade to witness the true potential of platelet derive products in dentistry leading to the evolution of PRP [3].

Platelet rich plasma is essentially an autologous collection of platelets suspended in a small amount of plasma obtained by centrifugation. "PRP" in short, is also synonymous with other terminologies such as autologous platelet gel, plasma rich in growth factors, as well as platelet concentrate [4].

Platelets concentrates

Platelet concentrates have three standard characteristics:

i) They act as scaffolds.

ii) They serve as a source of GF.

iii) They contain live cells.

(Figure. 1 describes the rationale of using platelet concentrates in tissue regeneration).

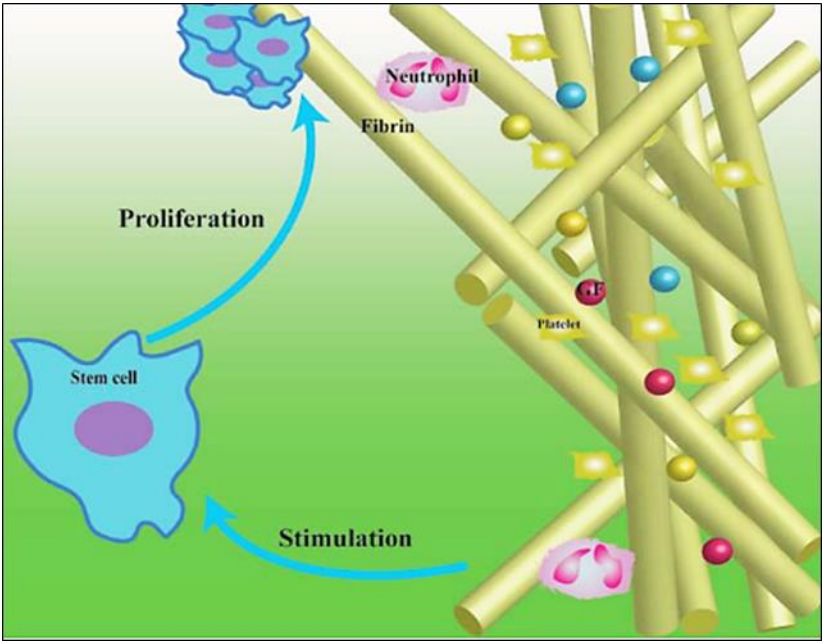

Fig 1: Schematic of the rationale of using platelet concentrates in tissue regeneration. GF, growth factor

These characteristics make platelet concentrates suitable candidates for tissue regeneration in clinical practice. The clinical applications of these types of derivatives of platelet concentrates in sports, spine and musculoskeletal medicine, ophthalmology and oral surgery have increased over the past few decades, resulting in positive clinical effects due to their ability to rapidly regenerate tendon, muscle and bone cartilage and speed up wound healing. Platelet concentrate injections in the knee for curing early osteoarthritis have achieved satisfying clinical results [5].

Platelet concentrates may serve as filling material for post-extraction alveolar sockets, resulting in increased bone quality and soft-tissue healing. In addition, dormant corneal ulcers associated with surgery may be healed by platelet concentrates prospectively. Chronic ulcers, characterized by a prolonged, self-perpetuating inflammatory phase, slow and defective formation of extracellular matrix and a decreased rate or a failure of re-epithelialization are general complications associated with diabetes or bedsores [6]. Platelet concentrates provide a novel standard of chronic wound care and achieve satisfying healing effects in the clinic. These positive results suggest that platelet concentrates may be used to effectively manage simple clinical issues.

However, the single use of platelet concentrates may not always provide satisfactory clinical results, particularly as complex clinical issues are concerned, such as substantial wounds accompanied with infection, where the high demands of wound healing result in a high degree of tissue tension. Occasionally, the goal is to restore tissues rapidly with improved healing results, such as in skin and cartilage, with the restored tissue being similar to the normal tissue, thus making it functionally better or compared with the simple use of platelet concentrates. In addition, prolonged degradation of platelet concentrates is required to keep pace with the slow regeneration of bones. Thus, it is necessary to develop platelet concentrates further, such that they are suitable for use in complex clinical applications and meet regenerative expectations. The present review highlights novel strategies utilizing platelet concentrates for tissue engineering and discusses several aspects of these strategies, such as lyophilized platelet concentrates, as well as the combination of platelet concentrates with biomaterials, stem cells or medications.

Methods

A comprehensive literature search was performed to identify appropriate studies for inclusion, incorporating several data-bases. and using permutations of multiple search terms as follows: Platelet Rich Plasma; PRP; Platelet Rich fibrin; Platelet Rich Fibrin; PRF; Platelet-Rich Fibrin; Leukocyte Platelet Rich Fibrin; Leukocyte Platelet-Rich Fibrin; LPRF; L-PRF; Advanced Platelet Rich Fibrin; Advanced PRF; A-PRF; APRF Injectable Platelet Rich Fibrin; IPRF; Concentrated Growth Factors; CGF and Soft Tissue Regeneration; Soft Tissue Wound Regeneration; Soft Tissue Wound-Healing; Wound Healing; Bone Regeneration; Bone Formation; Tissue Engineering. The inclusion criteria comprised studies published in the English language and both *in vitro* and *in vivo* evaluations of novel tissue regeneration applications of platelet concentrates. Methods, objectives, conclusions, potential future directions and potential shortcomings of included studies were reviewed.

Classification and preparation of platelet concentrates

Platelet concentrates may be defined as autologous biological products obtained following centrifugation of peripheral blood. Different centrifugal speeds result in various types of platelet concentrates. PRP was introduced in the 1970s and is recognized as the first generation of platelet concentrates [2]. PRP is produced by a two-step procedure: Blood is collected and centrifuged at high speeds after the addition of an anticoagulant. The plasma, erythrocytes and buffy coat separate at the top, bottom, and in the middle layer, respectively. The buffy coat contains platelets and leukocytes. Blood cells are

removed and the remnants are centrifuged at a higher speed. Finally, PRP is collected from the bottom layer. To obtain insoluble PRP, bovine serum or 2% of calcium chloride are added. This process converts plasmatic fibrinogen into insoluble fibrin scaffolds and activates the platelets. However, the primary concern with this procedure is the presence of chemical or biological additives that may have adverse effects on tissue regeneration and delay wound healing.

Consequently, in 2001, the optimization of the preparation protocol was introduced by Choukroun, a second generation of platelet concentrates called PRF, which was subsequently renamed as leukocyte PRF (L-PRF), an enhanced version of PRP [7].

PRF has three primary advantages:

i) Chemical or biological additives are absent, which avoids their related adverse reactions.

ii) Fibrin clots are naturally formed, so there is no requirement to add platelet activators, such as bovine serum or calcium chloride.

iii) A higher concentration of host immune cells is collected in the fibrin matrix.

Since low-speed centrifugation preserves more immune cells, GF and cytokines, this procedure has gained popularity, resulting in the production of advanced PRF (A-PRF) and injectable PRF (i-PRF). In a standard protocol, PRF is centrifuged at 708 x g for 12 min, whereas A-PRF is processed at 208 x g for 8 min and i-PRF is centrifuged at 60 x g for 3 min. Another advantage of i-PRF is that it produces a solid fibrin clot within 15 min, which gives operators adequate time to handle it and combine with other materials.

CGF is considered the third generation of autologous plasma and is prepared by a different centrifugation method. These platelet concentrates are produced by centrifuging blood samples at dynamic speeds, which increases the density of the fibrin matrix and the concentration of Growth Factor [8].

Rationale of using platelet concentrates in tissue restoration

The wound-healing process consists of five stages: Hemostasis, inflammation, cell proliferation, migration and tissue remodeling/maturation. Platelets, GF, leukocytes and fibrous proteins serve essential roles in these phases. The respective functions of the primary ingredient's plasma. In general, GF (Growth Factor) stimulates the proliferation of stem cells (Fig. 1). These factors include platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and transforming growth factor-β (TGF-β) [9]. They serve different roles in

different phases of the wound-healing process. The function of PDGF may be summarized as follows: First, it initiates and drives the wound-healing process by guiding neutrophils, macrophages and fibroblasts into the wound site from the early stage; subsequently, it activates macrophages and other cells possessing the potential for differentiation, such as fibroblasts, thereby resulting in provisional extracellular matrix synthesis, stem cell proliferation and collagen synthesis; finally, it triggers remodeling of the wound by activating and cross-linking collagen. Angiogenesis is a part of the tissue remodeling/maturation phase in wound healing. In this phase, both bFGF and VEGF promote endothelial cells to migrate and proliferate and differentiate into new blood vessels. Furthermore, TGF-β may promote angiogenesis, collagen synthesis and collagenase secretion and modulate osteoblastic cell division.

During the synthesis of autologous fibrin scaffolds, the 3-dimensional fibrin nano-scaffold incorporates the platelets in a non-diffusible mode and binds platelet-and plasma-derived GF before they attach to their corresponding cell-surface receptors. As the fibrin matrix is gradually degraded, GF and platelets are slowly released into the injured site at a rate consistent with that required for tissue growth. Furthermore, the fibrin matrix provides mechanical support, plastic-elastic stiffness and space for cellular migration and proliferation (Fig. 1). Thus, platelet concentrates fulfill different requirements for tissue restoration by linking molecular and cellular events.

Strategies to enhance the properties of platelet concentrates for clinical applications

General: The tissue regeneration process involves cells, signaling biomolecules and scaffolding. The reparative protocols introduced in the present review focus on the application of platelet concentrates in several forms, either lyophilized or blended with (bio)materials (chitosan, silk fibrin, metal nanoparticles, mineral trioxide aggregate graft materials and hydroxyapatite) and/or stem cells [adipose-derived stem cells, bone marrow stromal cells (BMSC) and human dental pulp cells] or those modulated with drugs (antagonism and synergism) to enhance tissue regeneration.

Lyophilized platelet concentrates: Lyophilization, a process comprising sublimation and desorption of water from the frozen sample, may improve protein stability, extend shelf- life and preserve the biological activity of samples. Therefore, although it is not necessary to use platelet concentrates after lyophilization immediately, there may be concerns regarding whether lyophilization affects the restorative abilities of platelet concentrates. Based

on the current body of knowledge, it is not easy to differentiate between the number of GF between lyophilized and fresh PRF. Furthermore, during the process of lyophilization, the structure of the fibrin matrix is altered substantially. For instance, the pore size of the fibrin matrix is enlarged, which accelerates its degradation rate. The process of lyophilization may also damage the structure of leukocytes and this may reduce immunological rejection. Since the amount of GF in lyophilized and fresh platelet concentrates retains consistency, the *in vitro* osteogenic capacity of BMSC sheets treated with fresh or lyophilized platelet concentrates is unaffected. Owing to the changes in the structure of lyophilize platelet concentrates, adhesion and migration of stem cells *in vivo* may in turn be affected, which may improve bone regeneration in bone defects compared with the fresh preparations [10]. However, results regarding lyophilized PRF are inconsistent. For instance, one study reported that lyophilized PRF appears to retain an architecture similar to that of fresh PRF (both lyophilized and fresh PRF exhibit an abundance of leukocytes with nuclei distributed within the fibrin matrix).

Platelet concentrates combined with biomaterials: Biomaterials are widely used in tissue engineering for their unique biological characteristics, which may be used to cover the shortcomings of platelet concentrates. Potential shortcomings of platelet concentrates include the possibility that released cytokines may not sustain tissue regeneration for a prolonged period and that platelet membranes may be vulnerable to mechanical stress, limiting their surgical applicability. Therefore, the following section addresses several approaches that have been proposed to overcome this problem.

Chitosan: Chitosan contributes to several phases of wound repair via the promotion of cellular organization. This biomaterial serves a role during proliferation and matrix generation, which may reduce the formation of disoriented connective tissue. Biocompatible membranes adsorbed with PRP and chitosan have excellent wound-healing effects. The composite scaffolds control the release of cytokines and enhance the proliferation of stem cells compared with platelet concentrates alone.

Silk fibroin: Silk fibroin offers good biocompatibility, slow degradation and excellent mechanical properties. When tissue defects are too large, platelet concentrates filling the defects may not be sufficient and in such cases, the combination of silk fibroin and PRF is a potentially suitable option. Owing to the excellent biocompatibility of silk fibrin, tissue regeneration may be accelerated by such synthetic biomaterials. However, slow degradation may induce immuno-reaction and has an adverse impact on bone formation, as the

scaffolds would occupy the space for bone formation. Thus, it is necessary to further explore the relationship between biodegradability and osteo-induction and calibrate the degradation rates of scaffolds for optimal bone formation.

Metal nanoparticles: Metal-derived nanoparticles are biocompatible, multifunctional and versatile materials, which are widely used as diagnostic and drug delivery tools in anticancer and antibacterial therapy. In the majority of cases, platelet concentrates are exposed to the oral cavity, where a large number of microorganisms may interfere with the treatment. Silver nanoparticles (SNP) have both high biocompatibility and antimicrobial properties. Preparation of platelet concentrates with the addition of SNP improved both the antibacterial abilities, primarily via the inhibition of viridans streptococci, and enhanced mechanical strength. A range of metal particles are being applied in medical research for different purposes and these nanoscale particles may endow platelet concentrates with novel biological properties. Appropriate nanoalloy particles not only improve the properties of platelet concentrates but also broaden the scope of clinical application.

Mineral trioxide aggregate (MTA): MTA is widely used in endodontic applications, including pulp capping, root perforation repair and root-end filling, to induce pulp regeneration and facilitate dentin bridge formation without causing inflammation. In 2016, Woo *et al*. [11] aimed to evaluate the effect of combined MTA and PRF on odontoblastic differentiation in human dental pulp cells (h-DPC). Indeed, the combination exhibited a synergistic effect by accelerating differentiation via activation of the bone morphogenetic protein (BMP)/Smad signaling pathway. Since both materials promote pulp and dentin regeneration, their combination may represent a novel therapeutic strategy in the context of pulpitis.

Graft materials: Graft materials are frequently employed to improve bone volumes for dental surgery, such as autografts, allografts, xenografts and alloplastic grafts. However, the effects of grafts on osteogenesis may be limited without the assistance of autologous bioactive ingredients. Of note, platelet concentrates may help overcome these issues. The bone regeneration capacity of PRF mixed with β-tricalcium phosphate is higher than that of PRF alone, although the difference is only significant in the initial two weeks post-surgery. Furthermore, there are discrepancies regarding the effects of PRF combined with different grafts on bone formation and maturation. Autograft-PRF exhibits outstanding results in the formation of new bone when compared with any other combinations and the xenograft-PRF combination is also promising for such applications. This may be ascribed to the abundance of bioactive BMSC in autografts, which may cooperate with platelet concentrates

to actively regenerate bone tissue. In addition, autografts may reduce immunological rejection compared to other types of grafts. In general, platelet concentrates provide a plethora of bioactive molecules, which may promote bone regeneration and deposition in graft materials.

Hydroxyapatite (HA): HA is a calcium phosphate compound that serves as a bone stuffer due to its mineral composition being similar to that of bone tissue. However, its use in surgery is limited owing to the challenge of handling HA at operation sites. Consequently, it is necessary to deliver HA granules using biomaterials. A liquid platelet concentrate may be appropriate for carrying HA granules. After thoroughly mixing the HA with platelet concentrates, the blend may be solidified prior to being implanted into tissue defects. On the other hand, fibrin glue and activated PRP loaded on composite scaffolds based on chitosan-gelatin/nanohydroxyapatite may improve osteogenic differentiation and proliferation of dental pulp stem cells. However, synthetic scaffolds offer certain advantages. First, platelets are typically scattered on the surface of the membrane, suggesting that the release of GF is smooth. In addition, the fibrin glue may guide the stem cells to adhere to the network structure and stimulate cells without further migration, which limits mesenchymal cell mobility. Finally, the scaffold provides a microenvironment rich in GF to promote differentiation and proliferation of stem cells. Both fibrin glue and PRP may promote the synthesis of the extracellular matrix.

Platelet concentrates combined with stem cells

Adipose-derived stem cells (ADSC). ADSC have a high potential to differentiate into several types of cells. In addition to their multipotent differentiation ability, the paracrine functions of ADSC also influence tissue regeneration. Certain studies suggested that the ability of ADSC to repair tissues originates from stimulating collagen synthesis, cellular matrix protein synthesis and dermis revascularization. The scaffolds and GF in platelet concentrates favor the proliferation of ADSC. It may thus be assumed that the combination of platelet concentrates and ADSC enhances tissue regeneration. PRP functions co-operatively with conditioned medium obtained from adipose-derived MSC (ADMSC) to promote the proliferation and migration of fibroblasts and keratinocytes *in vitro* [12]. It is assumed that PRP is able to interact with the conditioned medium from ADMSC to exert beneficial effects. However, although conditioned medium stimulates proliferation in a concentration-dependent manner, excessive PRP concentrations may have adverse effects on proliferation. This may be attributed to GF-inhibiting proteins within the complex mixture produced by platelets (such as platelet

concentrates containing high levels of proteolytic enzymes inhibiting cell growth). Furthermore, a deeper association between the activities of different cells was determined; a low concentration of PRP may promote the proliferation and migration of ADMSC and fibroblasts. In addition, the paracrine activity of ADMSC stimulates keratinocyte proliferation. Thus, there is a promising prospect for healing of cutaneous ulcers by exploiting the reparative synergy between ADMSC and PRP in the clinic.

It is particularly challenging to re-establish a sufficient blood supply after maxillofacial soft-tissue defects caused by irradiation. This leads to difficulties in recovering the damaged tissue to its pre-damaged condition both in terms of function and aesthetics. The abundance of GF in platelet concentrates and the multipotent differentiation potential and paracrine ability of ADSC provide an opportunity for healing this kind of tissue damage. A combination of platelet concentrates and stem cells induces adequate angiogenesis and reduces apoptotic activities.

BMSC: BMSC serve important roles in osteogenesis. It is possible to combine BMSC with platelet concentrates to produce bone tissue. The combined autologous BMSC and PRP may promote structural allogenic bone graft healing in a rabbit bone defect model. Abundant bridging trabeculae and increased expression of relevant osteoblastic proteins were determined in BMSC and PRP, suggesting the effectiveness of this combination. Periodontitis, a widespread chronic disease, destroys both soft and hard tissues. With traditional treatment, it is hard to regenerate the complete periodontium, including periodontal ligament (PDL), cementum and alveolar bone. Loading the PDL and jaw bone MSC sheets on PRF to restore periodontal defects is a novel approach. The two above-mentioned stem-cell types were used to form cell sheets that were able to differentiate into PDL- and bone-like tissues, respectively. Furthermore, PRF was used as a 3-dimensional fibrin network to support, sustain and nourish the cell sheets in the space between the simulated root surface and alveolar bone. After the implantation, PDL and bone-like structures were successfully induced in corresponding sites. It is not easy for articular cartilage to regenerate after injuries, even though progenitor cells are present in the superficial zone. Though autologous chondrocyte implantation is an established strategy for repairing cartilage injuries, there is a chance that fibrocartilage may form. In addition, pure BMSC injection leads to a small proportion of stem cells attaching to the defect sites to repair the injured cartilage, which may lead to unsatisfactory cartilage restoration. However, BMSC injection combined with PRF releasates offers improved results. PRF releasates may increase the

thickness of the regenerated cartilage and improve the histological morphology, which may be ascribed to the roles of GF. Thus, the combination of PRF releasates and BMSC highlights an alternative strategy for autologous chondrocyte implantation to cure cartilage defects.

Stem-cell treatment has gained popularity in recent years. It is a feasible means of combining stem-cell treatment with platelet concentrates, as the platelet concentrates provide scaffolds for cells to adhere to and migrate from and the GF may promote stem-cell proliferation. In addition, the activities of different types of cells may synergistically interact to produce beneficial outcomes. h-DPC. Dental pulp regeneration is an important aspect of recovery from pulpitis and periapical disease. Therapeutic assistance, aimed at facilitating such regeneration, currently involves the use of differentiated cells, biological scaffolds and GF. There is an urgent requirement to develop more appropriate biocompatible scaffolds. Addition of an h-DPC suspension to blood prior to centrifugation during PRF preparation may be a feasible approach. This method distributes h-DPC to the buffy coat along with leukocytes, preserving h-DPC viability. This is because h-DPC are naturally occurring human cells, such as leukocytes.

Combination of platelet concentrates with drugs

Antagonism: Certain medications used during the treatment process may cause adverse effects. For instance, bisphosphonates are used to manage osteoclast-mediated osseous resorption. However, long-term administration of bisphosphonates may result in a rare but adverse complication, namely osteonecrosis of the maxillary and mandibular bones. This side-effect may be ascribed to cytotoxic effects on osteo-blasts, which may prevent cells from proliferating, adhering and migrating. Different types of platelet concentrates may be able to inhibit bisphosphonates on different levels. However, PRF may have advantages over PRP in promoting stem-cell proliferation. This discrepancy may be due to two reasons. First, the release of GF from PRP occurs more rapidly than that of PRF due to the natural scaffold in PRF. Furthermore, a higher concentration of leukocytes in PRF may positively influence cell proliferation and migration, although the function of leukocytes is disputed. The plant-derived stilbenoid resveratrol has anti-inflammatory, antioxidant and bone-protective properties. In addition, it may enhance the therapeutic outcomes of CGF treatment in bisphosphonate-related osteonecrosis of the jaw. In particular, resveratrol may modulate the effects of CGF to protect osteoblasts. However, the influence of resveratrol on CGF against bisphosphonate-induced osteonecrosis is not always synergistic, as the outcome is dependent on experimental conditions.

Synergism. As mentioned above, engineered adipose tissues have recently gained popularity for use in reconstruction of soft-tissue defects. However, adipocytes are fragile and it is difficult to preserve them after grafting due to an inadequate blood supply. Ginsenoside Rg1 (G-Rg1) and PRF have been used to pretreat human breast adipose-derived stem cells (HBASC) prior to being seeded in a 3-dimensional porous sponge scaffold of collagen type I (84). G-Rg1 and PRF may induce the expression of angiogenic factors and G-Rg1 may increase the paracrine activity of HBASCs. Therefore, it is reasonable that the combination of G-Rg1 and PRF may result in the production of more adipose tissue than that produced by any material alone. Statins may increase BMP-2 expression directly to promote bone formation and may modulate the activities of osteoclasts and osteoblasts. PRF, an autologous fibrin scaffold, may not only serve as a carrier for statins, but also enhances bone formation due to its biological properties. The combination of PRF and statins provides superior osteosis, upregulation of both BMP-2 and VEGF protein and elevated bone anabolic serum markers as compared with those in samples treated with simvastatin or PRF separately.

Reasonable combinations of platelet concentrates with drugs may reduce side effects and enhance the effects of drugs due to the mutual effects between drugs and cytokines in platelet concentrates. Furthermore, the fibrin matrix scaffold may serve as carriers for drugs in the target location. Thus, it is recommended to implement a combination of platelet concentrates and drugs to solve clinical issues.

Role of platelet concentrates in tissue regeneration in periodontology

In 1974, it was discovered for the first time that platelets could regenerate [13]. Platelets, which are tiny blood cells, carry out numerous physiological processes. They are crucial to preserving adequate blood volume in persons with vascular injury due to their clotting activity and stimulation of the coagulation factors [13]. The alpha granules are immediately released after platelet activation and contain large sticky proteins like VWF, TSP1, vitronectin, and fibronectin, mitogenic factors like PDGF, VEGF, and TGF, coagulation factors like factors V, VII, XI, and XIII, and protease inhibitors like protein C, PAI-1, and TFPI. Platelets are produced by megakaryocytes in the bone marrow. 9 According to Schwertz *et al*., [14] platelets from sites other than the bone marrow have been found to promote tissue regeneration. The ability of platelets to clot blood is widely known. Platelets function in inflammatory processes, angiogenesis, cell proliferation, and differentiation in addition to stopping bleeding.

PRF Over PRP

When compared to PRP, PRF has a number of advantages, including lower levels of polymerization, simplicity of manipulation, no biochemical processing of patient blood samples, support for the immune system, and comparatively better wound healing capacity. Utilizing the PRF has less risks than utilizing PRP since it lacks anticoagulants and bovine thrombin and is more effective at promoting cell migration and proliferation. Due to its beneficial qualities, which are outlined below, the PRF has been the subject of several studies looking at its potential for usage in various tissue regeneration applications in dentistry [15].

Conclusion

Autologous platelet-derived concentrates are biocompatible products containing cytokines, platelets, leukocytes and fibrin, and may be regarded as a slow-release system, which ensures sustained delivery of active principles and GF for ~2 weeks. Of note, the release kinetics may influence the entire process of tissue regeneration. However, certain problems remain that must be addressed to optimize the formulation intended for tissue regeneration. The freeze-drying method is an effective method for preserving the viability of platelet concentrates for comparatively longer periods, without any concerns regarding the loss of their effects in tissue regeneration. Combination with biomaterials, such as chitosan and silk fibrin, may enhance physical strength, making it suitable for suture in surgery. The synergistic effects of platelet concentrates and bone regenerative materials, including HA and graft materials, may induce rapid bone regeneration in diseases associated with bone loss, such as periodontitis and bone defects caused by tumours, amongst others. The addition of stem cells provides a novel approach for the rehabilitation of tissue morphology. Particularly in the skin and cartilage, it is necessary to restore the defective tissue to normal levels, which is important for aesthetics and normal function. Due to the complexity of clinical issues, tissue defects associated with infection and tissue damage caused by side effects of drugs are common in the clinic. Nanometal particles, such as nano-silver, have significant anti-bacterial properties. Combination of nano-silver and platelet concentrates provides integrated methods to inhibit bacteria and promote tissue regeneration for patients with burn damage. The antagonism of platelet concentrates to osteonecrosis induced by long-term administration of bisphosphonates provides a suitable method for curing patients with osteonecrosis. The efficacy and suitability of these creative approaches require to be further verified in clinical trials. Certain limitations remain in the field of tissue regeneration using platelet concentrations as follows:

i) No studies are currently available on the application of modified methods using platelet concentrates, to the best of our knowledge, and thus, modified methods that may be used in the clinic were not reviewed.

ii) Particularly the underlying mechanisms remain elusive.

In the future, studies on the detailed mechanisms of modification should be performed and evaluation of these experimental methods in the clinic should be performed. The use of platelet concentrates has demonstrated positive clinical effects in several fields of regenerative medicine. Simple administration of platelet concentrates cannot always meet the complex demands of clinical issues. Furthermore, different types of tissue have different healing periods. Future studies focusing on the methods of establishment of platelet concentrates as a multifunctional medical procedure, meeting the complex clinical issues, are thus required. In addition, the properties of platelet concentrates are important for clinical use, such as the ability to tightly bind with the tissue-surrounding defects and strong resilience, avoiding disruption. Thus, additional studies on modified strategies using platelet concentrates and prospects for clinical use are required.

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Chapter - 7
Dental Implant Related Complications and its Management

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Chapter - 7

Dental Implant Related Complications and its Management

Dr. Hema Kanathila and Dr. Suvidha Patil

Abstract

Replacement of lost teeth with dental implants is in great popularity. Despite recent advances, implant therapy still requires a biological healing and integration phase. Implant related complications and failures can occur due to many reasons. It can occur either during surgical phase or in the 2nd stage or in the prosthetic phase. Dental implant related complications and failures encountered by the implant specialists can be managed by a detailed knowledge on the management of these complications and failures. Most of the time, complications can be avoided with thorough attention to diagnosis, treatment planning, and well-executed surgical and prosthodontic procedures. This chapter concentrates on various dental implant complications and its management to achieve a successful treatment outcome.

Keywords: Dental implant, complications, implant failures, periimplantitis, implant fracture

Introduction

Dental implants have been employed for decades to replace lost teeth. With the increased use of dental implants in order to replace lost teeth, there has been an increase in complications. The survival statistics of implants refer to prostheses that have continued to provide clinical service during a specified follow-up time, and this does not necessarily imply that they are free of problems. Dental implant issues can range from surgical complications to aesthetic complications. Early failures are typically connected with surgical trauma that results in failed osseointegration, whereas late failures occur after prosthetic restorations are completed. When a failure occurs, the decision-making tree must be used to help and streamline the process of choosing the best alternative. As a result, understanding the various variables leading to the complications and how to manage them becomes critical. The management of patients who have specific risk factors and the ability to negotiate possible complications and failure are critical skills for implant surgeons and dental professionals.

Despite recent developments, implant therapy still entails a biological healing and integration process. Dental implant failure has been linked to risks and consequences, despite ongoing advancements in implant systems and alternative interceptive treatment approaches. Due to the fact that risk factors are frequently to blame for implant failures, they demand urgent attention. Systemic disorders, age, sex, maxillary implant site, smoking, quantity and quality of bone are among the statistically analysed factors linked to implant failure.

The risk factors are described as

Age

One of the key prognostic criteria for implant success is considered to be age. Because of the age factor, elderly individuals have altered systemic health conditions, prolonged healing time and bone conditions. Studies have proved that patients with age more than 60 have unfavourable outcome with low implant survival rate.

Systemic factors

Complications with implants are frequently caused by incorrect diagnosis and patient history, particularly with regard to systemic disorders. Some systemic factors like smoking, diabetes and radiation therapy have increased risk of bone loss. Other conditions like osteoporosis, scleroderma, Sjogren's syndrome, Parkinson's disease, and multiple myeloma pose high risk for implant failure. Patients with hormone replacement therapy, corticosteroid therapy and HIV-positive patients also pose high risk of implant failure.

Anatomic factors

Implant survival was not significantly impacted by either implant location or whether upper or lower jaw. Whereas, poor bone quality and severely resorbed jaw can lead to implant failure.

Microbial factors

Peri-implantitis is one of the leading causes of implant failures. *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans*, *Staphylococcus aureus*, *Prevotella nigrescens*, and *Fusobacterium nucleatum* are the commonly involved organisms in peri-implant inflammation and infection.

Occlusal loading related factors

Greater frequency of implant loss is seen in patients with bruxism than in those without parafunctional habits. The increased failure risk among patients

with parafunctional habits is caused by uncontrolled functional loading of the implant, which leads to micro-motions above the critical limit. This causes fibrous encapsulation instead of osseointegration hence leading to implant failure.

Another important factor that can lead to failure is the opposing occlusion or dentition. The patients with the highest implant failure rate were those whose implants opposed unilateral occlusal support. Occlusal overload brought on by using a smaller number of implants is another potential reason for implant prosthesis failure.

Charles Goodacre *et al*., had classified implant complications encountered by the dental specialists as implant loss associated to type of prosthesis and arch, duration after placement, bone quality, implant length, any surgical complications, peri-implant soft tissue complications, marginal bone loss, mechanical complications, and phonetic and esthetic complications.

Complications and management associated with implant

In dentistry, implant surgery is a common surgical treatment that is regarded as safe and having a high success rate. Dental implant surgery, like any other surgical procedure, can have a number of complications. Surgical complications can be treatment plan related, anatomy related or procedure related with patient selection being a crucial element in deciding success. In order to foresee issues before they arise, implant treatment planning should start with a review of pertinent medical history data and the identification of any potential contraindications.

Preoperative complications

Diagnostic Complication and Management

A detailed knowledge of the implant complications and their management is essential, which ensures prompt management and thereby ensures implant success. In case of any systemic disorders and medication-related complications, proper case history and diagnosis, systemic evaluation should be noted. Some diseases like myocardial infarction, diabetes, Parkinson disease, osteoporosis, cerebrovascular disease, smoking, cancer therapy and immunodeficiency can lead to complications during the implant treatment.

- **Osteoporosis:** Implant osseointegration may be impacted by osteoporosis's reduced bone metabolism.
- **Myocardial infarction:** The process of osseointegration is disrupted by changes in blood and oxygen supply.

- **Bleeding disorders:** Uncontrolled bleeding can result from the use of anticoagulant medications like warfarin and aspirin, platelet problems, and coagulant factor deficiencies.
- **Paget's disease:** Dental implant surgery may not be appropriate for patients with Paget's disease due to their reduced bone density.
- **Patient‘s with radiation therapy:** Seventy percent of dental implant placements in irradiated bone are successful. Implant success rates is increased in irradiated patients when hyperbaric oxygen therapy is administered before implant therapy.
- **Diabetes:** Hyperglycemia affects osseointegration of implants. After osteotomies, hyperglycemia can impede bone healing by 40%.
- **Parkinson's disease:** Poor motor control seen in Parkinson's disease can cause implant failure due to improper maintenance of oral hygiene.
- **Organ transplantation:** Individuals receiving organ transplants must take immunosuppressive drugs for an extended period of time in order to avoid organ rejection. Cyclosporine which is given usually in such cases can affect the bone healing around implant.
- **Smoking:** Smoking causes various problems leading to dental implant failure. Smoking delays healing and it causes decreased calcium absorption. It as well slows down blood flow because it increases peripheral resistance and platelet aggregation, that both slows down the osseointegration process.

Seek medical help if any emergency encountered during procedures, and maintain all life-saving drugs handy. Post-operative medications and regular recalls should be considered.

Post-operative complications

Evaluating and diagnosing the problematic implant is the first step in treatment. The clinical signs and symptoms of implant failure could include mobility, oedema, pain, pus, bleeding, and radiological substantiation of peri-implant bone loss. Early and late implant failure of implants are distinct categories. Early implant failure occurs when an implant shows clinical movement before to the implantation of a final prosthesis, and late implant failure happens one to three years following implant implantation. The most crucial step in the scenario that an implant fails or is unable to develop bone around it is its prompt removal to prevent further bone loss. Treatment options for implant failure is the replacement of a failing implant with one that has a

larger diameter or simultaneous replacement of a failing implant with a guided bone regeneration (GBR) procedure, and a staged or delayed method where the lost tissue is restored first and then implant is placed after the site has healed.

Biological complications and Management

Bacterial colonisation can be due to poor oral hygiene, microscopic gap between implant components or due to cements left behind in the subgingival region. This bacterial colonisation and plaque around the dental implants can cause Periimplantitis. Peri-implant diseases are more common in those who have had periodontitis. Hence, maintenance and supportive therapy such as mechanical debridement, antiseptic treatment, antibiotic treatment and regenerative or resective therapy should be considered and provided at least once a year, depending on the patient's past dental history and susceptibility to periodontitis. Pain, bleeding, movement of the implant are the signs of Periimplantitis. This can be managed by surgical intervention, non-surgical intervention or by using Lasers.

Fig 1: Comparison of healthy gums and peri-implantitis

Non-surgical intervention can be done by mechanical cleaning of the implant surface using prophy jets, steel or titanium curettes. Perio Chips, chlorhexidine lavage, jet devices, metronidazole gel, and slow release Ligosan are among the local medications that should be administered. Another modality of non-surgical treatment for periimplantitis includes the use of Lasers. Carbon dioxide lasers and Erbium-yttrium-aluminium-garnet lasers may be used for photodynamic therapy as well as decontamination in such cases. Whereas, in surgical management of periimplantitis case, a surgical flap is used to completely debride and clean the damaged implant.

Technical complications and management

Implant fracture is another pernicious complication, which the dental practitioner must pay attention to. It is a late biomechanical complication and is more in partially edentulous jaw than edentulous jaw. Defects in the raw materials or manufacturing process can lead to implant fracture. Other causes of implant fracture can be bone loss, excessive occlusal load or any patient related factors.

Management options which are considered can be complete removal of fractured implant, removal of only coronal part of implant to place new prosthetic components or removal of fractured coronal part leaving apical part fused to the bone. It requires the removal of implant components or rearranging of the implant's remnant in case where the implant is close to a crucial structure. In cases where it is close to a neurovascular bundle or sinus, it may be wise to rearrange the implant's remnant to allow tissues to cover it or allow tissues to repair over it if the implant was broken at a very low level.

Another way to remove the fractured implant is apicoectomy, an effective method where we replace new implants while simultaneously removing broken ones. In order to remove the apical fragments of the broken implant using this technique, a hole has to be made in the bone and a new implant is put in as usual, and the previously removed patient's own bone is used to substitute the hole. The fractured component can also be removed using trephines, after which a new implant can be placed. The entire removal of fractured implant is considered as the most apt solution in such cases. Whereas, in case of fracture of implant, another option is to remove the coronal portion, leaving the apical remnant, followed by treatment with conventional prosthodontics like fixed partial denture or removable partial denture. A prosthetic post should only be taken into consideration if there is still enough internal thread retention. It is usually preferable to make a better treatment planning by avoiding cantilever extensions in the posteriors and providing occlusal guards in patients with parafunctional trait to prevent this ruinous event of implant breakage.

Fig 2: Implant fracture

Studies have shown that single implant restorations in the posterior maxilla and mandible loosen by 38%. As a preventive measure, it is better to tighten the implant screws again 10 minutes later to the initial torque application in order to lessen the settling effect. Use of mechanical torque gauges and sealers to close up the spaces between these screws and the implant threads and adhesives to enhance frictional resistance can be helpful in diminishing screw loosening problem. Studies on screw loosening issue has recommended a few measures to be considered like positioning of implant perpendicular to occlusal plane, using components with anti-rotational features and by using frameworks with minimum cantilever lengths.

In case of abutment screw fracture, artery forceps can be used to unscrew the fragmented portion of the implant, if it is above the head of the device. In case where the fractured part cannot be grasped, ultrasonic scaler can be used which can help in disengaging the threads by its oscillations. It can also be managed by using a 1/4 round bur at a counter-angle low speed while operating in reverse to spin the screw out. Cases where the abutment screw fracture is deep, the repair kit can be used.

Guided bone regeneration has been a boon in cases where there is deficient bone volume. Membrane exposure to oral environment and wound dehiscence can lead to implant loss and treatment failure, at the cost of both patient's and dentist's time and effort. A rare observation during postsurgical follow-up is an exposure less than 3 mm without any purulent exudation, which does not create any signs or symptoms in the patient. If the exposure is more than 3mm, the membrane has to be removed so as to prevent infection. In case of any purulent discharge, the membrane has to be removed and then the graft must be removed which can hamper the healing process. An antibiotic coverage is also necessary.

Aesthetic complications and management

The aesthetics of any prosthesis take precedence because all in the present world are concerned with appearance and demand the same. Malposition of implant in the aesthetic area is one of the key contributors to aesthetic challenges in the domain of implant dentistry. Proper 3D placement of implants is crucial for implant therapy to produce the best aesthetic and functional benefits. A surgical guide should be considered if it is expected that it will be difficult to position the implant correctly. Surgical stents are especially recommended in situations where there are several missing teeth. The orthodontic bone stretching (OBS) approach, which combines strong orthodontic forces with deep partial osteotomies, may be used to address implant malpositions. By shifting the alignment of the gingival line and implant axis towards the occlusal plane, the applied force aids in the rehabilitation of aesthetics.

Fig 3: Aesthetic failure of Dental Implant treatment

Another complication to mention is the loss of implant or graft material into the maxillary sinus, which can affect the ciliary movement in the sinus and mastication. This must be surgically managed using a variety of approaches, such as endoscopic, intraoral, transnasal and reconstruction of maxilla.

Rarely implant may have to be removed due to different reasons like loose implants, nerve damage or severe peri-implantitis. A few procedures for removing immobile implants include the use of forceps, counter-torque ratchets, elevators, piezo tips, screw removal tools, high-speed burs or trephine burs. In some cases, in order to avoid the harm to the surrounding tissues, counter-torque ratchet technique (CTRT) or the reverse screw technique (RST) may be used.

Ingestion/Aspiration and Management

If aspiration occurs, catastrophic repercussions may result. Preventive measures like using throat gauze screens and floss ligatures on implant should be encouraged. A patient should be taken to the hospital if they swallow or aspirate an implant component since acute blockage can be fatal and delaying the removal of foreign objects may make bronchoscopy more technically challenging.

Implant maintenance

In addition to the dentist's clinical role, the patient and the hygienist are crucial to the success and care of implants. Sonic and ultra sonic scalers with special attachments like nylon sleeves and plastic attachments are used for scaling around dental implants. Plastic and Teflon coated curettes can be used sub gingivally without changing the topography of dental implants.

Manual tooth brushes, mechanical tooth brushes, dental floss, interproximal brushes and antimicrobial mouth rinses should be used as home care by the patients for the maintenance of dental implants which will help in achieving a successful treatment outcome for a longer period of time.

Conclusion

The "planning" steps of an implant should take some time. The dental practitioner must always review and keep in mind the fundamentals of anatomy. The knowledge and awareness of the complications related to implant and their management is very important for dental practitioners for better evaluation, mapping out a more predictable treatment plan and to achieve a good clinical outcome in implant dentistry. For long term success, the preservation of the healthy tissues around an implant is one of the most important aspects. A thorough oral hygiene has to be followed by the patient with regular follow-ups.

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Chapter - 8
Dental Management of Patients with Cardiovascular Diseases

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Chapter - 8

Dental Management of Patients with Cardiovascular Diseases

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Abstract

Medical emergencies happen unexpectedly, so one has to be prepared for an emergency before it happens. When an emergency occurs in the hospitals, clinics, or in home, the first few minutes can make the difference between life and death. When the dentist sets priorities for preventive measures, an understanding of the relative frequency of emergencies and knowledge of those likely to produce serious morbidity and mortality are important. Recognition, prevention, preparation, basic life support, cardiopulmonary resuscitation, and specific medical emergencies are the priorities for the prevention of medical emergencies in dentistry. The risk of mortality or serious morbidity can be reduced by ensuring the basic emergency equipment and medications are present in the dental setup and the dental team should be well trained in basic life support measures.

Keywords: Emergency, dental procedure, cardiovascular, syncope, drugs

Introduction

Cardiovascular diseases (CVD) comprise of a group of diseases of the heart and vascular system affecting majority of individuals worldwide. Ischemic heart disease, Hypertension, Dysrhythmias, and Infective Endocarditis are some of the cardiovascular conditions most commonly seen among the population. Co-existent cardiovascular disease is the most frequently cited medical condition for patient referral from general dental practitioners to hospital departments, which reflects widespread concern over potential problems during treatment. Dentists must be able to identify medical emergencies and adopt the opportune measures to avoid them or treat them quickly and effectively. A comprehensive treatment plan should be constructed keeping in view all the pros and cons related to the patient's medical condition.

Relationship of cardiovascular disease and periodontitis

Periodontitis has been proposed as having an etiological or modulating role in cardiovascular and cerebrovascular disease, diabetes, respiratory disease, and adverse pregnancy outcome and several mechanisms have been proposed to explain or support such theories and oral lesions are indicators of disease progression and oral cavity can be a window to overall health and body systems. One of these is based around the potential for the inflammatory phenomenon of periodontitis to have effects by the systemic dissemination of locally produced mediators such as C-reactive proteins (CRP), interleukin-1b (1L-1b) and interleukin-6 (1L-6), and TNF-a.

Another indirect effect of periodontal infection that may explain the association between periodontal disease and heart disease is that periodontal organisms contain proteins which cross-react with the heart. The heat-shock protein-60, which is produced by Tannerella forsythia and Porphyromonas gingivalis, has about 60% homology with the mammalian heat shock protein. It is known that antibodies to the heat-shock protein are found in patients with periodontal disease. These antibodies to heat-shock proteins of periodontal bacteria are cross-reactive with the heat-shock protein that is exposed in an injured endothelium or atheromatous plaque. This could set in motion autoimmune phenomena and contribute to atheroma formation. There also may be common genetic mechanisms which provide the link between periodontal disease and cardiovascular disease.

Angina pectoris

Angina is transient chest pain resulting from temporary inadequate blood supply to (and thus inadequate oxygenation of) the heart, usually secondary to narrowing, blockage, or spasm of one or more coronary (heart) arteries. It is not a myocardial infarction (in which heart muscle dies), but rather a phenomenon that signifies increased risk of myocardial infarction (MI), cardiac arrest, or sudden cardiac death.

Anginal chest pain may be precipitated by physical activity, exercise, emotional stress, periods of extreme cold or hot temperatures, heavy meals, drinking alcohol, or smoking. Other causes include uncontrolled high blood pressure, aortic stenosis (narrowing of the aortic heart valve), and hypertrophic cardiomyopathy (enlarged heart).

Symptoms of angina include: [3, 4] sharp, burning, or cramping pain that starts in the central chest and then radiates to the left arm, neck, back, throat, or jaw; pressure, tightness, squeezing and/or aching feeling in the chest or arm(s); retrosternal burning sensation similar to persistent severe

gastroesophageal reflux; ache that starts in the neck, jaw, throat, shoulder, back, or arm(s); discomfort in neck or upper back, especially between the shoulder blades; and numbness in the arms, shoulders, or wrists.

During acute anginal episodes, the patient/client may be apprehensive and diaphoretic (sweating heavily). Heart rate is usually elevated, as is blood pressure. Respiratory difficulty (dyspnea) and a feeling of faintness may also occur.

Although most anginal episodes resolve without residual complication, myocardial infarction may occur (particularly in unstable angina), as may acute cardiac dysrhythmias.

Stable angina, the most common of angina, follows a predictable pattern of chest pain (lasting 1 to 15 minutes, and typically less than 10 minutes) and is usually relieved with rest or medication (e.g., sublingual nitroglycerin). Unstable angina is characterized by chest pain that is unpredictable, may happen at rest without precipitating physical activity, may be prolonged (up to 30 minutes), and is not readily relieved by nitroglycerin. Variant angina (caused by coronary artery spasm) usually happens at rest (particularly between midnight and 8 a.m., often awakening a person from sleep) without precipitating physical exertion of emotional stress. Microvascular angina (caused by improper functioning of coronary microcirculation) manifests as chest pain without any apparent blockage in a coronary artery. Atypical angina presents without the typical chest symptoms of angina, and instead may manifest as vague chest discomfort, shortness of breath, back or neck pain, retrosternal burning, or fatigue.

Anginal symptoms in women can be different than classic angina symptoms. For example, females often experience nausea, abdominal pain, extreme fatigue, or shortness of breath with or without chest pain. Discomfort in the neck, jaw or back may occur, and chest pain, when present, may be stabbing in nature rather than the more typical pressure-like sensation.

Risk factors for angina (and coronary artery disease in general) include diabetes mellitus, hypertension, tobacco use, high blood cholesterol or triglyceride levels, older age (greater than 45 years in men and 55 in women), inactive lifestyle, obesity, and stress. Hyperthyroidism and severe anemia can also trigger anginal chest pain.

Oral manifestations

There are no oral findings specific to angina (or coronary atherosclerotic heart disease in general); however, medications used to treat angina can result in oral changes.

Gingival hyperplasia can be caused by calcium channel blockers (e.g., amlodipine, nifedipine, felodipine, verapamil, and diltiazem).

Taste changes and lichenoid lesions can result from beta-blocker use (e.g., metoprolol and propranolol).

Lupus-like oral and skin lesions can result from use of direct vasodilators (e.g., hydralazine).

Bleeding-manifesting as petechiae, hematomas, or gingival bleeding-may occur in patients/clients receiving antiplatelet or anticoagulant treatment.

Dental management of angina pectoris

Similar to patients/clients with a previous myocardial infarction (MI), general management strategies for patients/clients with stable angina include the following: short appointments, comfortable chair position, pre-treatment vital signs, and availability of nitroglycerin and oxygen in case a medical emergency should arise. The patient/client should be engaged to determine the best time of day for an appointment, which is often the morning when the patient/client is well rested and has a greater physical reserve. A stress reduction protocol may be utilized where indicated.

All dental/dental hygiene procedures should be stopped with the onset of chest pain. The patient/client should be allowed to position himself/herself in the most comfortable position, which is usually sitting or standing upright. A member of the emergency team should immediately get the emergency kit and oxygen. Nitroglycerin should be administered sublingually (i.e., tablet, often the patient/client's own) or transmucosally (i.e., spray)1. Emergency medical care should be sought if chest pain is not relieved by 3 nitroglycerin tablets or spray doses in conjunction with oxygen over a 10-minute period. In a person with previously unrecognized coronary disease (i.e., no history of angina), chest pain persisting more than 2 minutes is an indication for emergency medical assistance.

After termination of an intra-office anginal episode, the factor(s) that might have caused it to occur should be determined. This will inform modification of future dental/dental hygiene treatment.

While dental/dental hygiene treatment may resume at any time after termination of intra-office anginal pain in patients/ clients with stable angina (including at the same visit if considered necessary), the patient/client should be allowed to rest until he or she is comfortable before resuming dental/dental hygiene care or discharge. (If unstable angina is suspected, treatment should not be resumed). Vital signs should be monitored and recorded before

discharging the patient/client. In the situation where doubt persists about the degree of recovery, medical assistance or consultation should be sought, or a friend or relative should be contacted to serve as an escort.

Patients/clients with gingival hyperplasia (secondary to calcium channel blocker [CCB] use) will likely require more frequent cleanings and enhanced dental hygiene education. Gum surgery may occasionally be required.

In addition to medical treatment, many persons experiencing angina undergo revascularization by coronary artery bypass surgery or increasingly by percutaneous coronary intervention (i.e., PCI: angioplasty + stent). To avoid stent thrombosis, patients/clients are typically placed on dual antiplatelet therapy (e.g., ASA [aspirin] and clopidogrel) following stent placement in the coronary arteries. For both types of stents, ASA is generally continued for life, and there is virtually no dental hygiene indication to discontinue such ASA treatment.

If the dental hygienist is concerned about potential peri-procedural or post-procedural bleeding, the patient/client's cardiologist should be contacted regarding the patient/client's antiplatelet regimen and optimal patient/client management should be discussed before discontinuing the antiplatelet medications. Such medications should not be discontinued prematurely given their importance in minimizing the risk of stent thrombosis, and discontinuation of these agents before dental hygiene procedures usually is unnecessary. However, if there is a concern that excessive bleeding may occur, clopidogel may have to be stopped several days before dental hygiene care that involves tissue manipulation; medical consult/clearance should be sought in this regard. If scaling procedures are planned for a patient/client on the anticoagulant warfarin, the patient/client's physician should be contacted to confirm that the PT ratio (prothrombin time) will be two times normal or less, or international normalized ratio (INR) is less than 3.0.

Non-steroidal anti-inflammatory medications (NSAIDs) should generally be avoided in patients/clients with established cardiovascular disease (particularly COX-2 selective NSAIDs such as celecoxib). Of the non-selective NSAIDs (which include ibuprofen), naproxen may be safest, but a physician should be consulted before use. Acetaminophen is usually preferred as an oral analgesic in patients/clients with angina.

Vasoconstrictor use (e.g., epinephrine in local anesthetic) poses potential problems for patients/clients with ischemic heart disease, including those with angina. Vasoconstrictors should be used sparingly, if at all, in dental procedures for patients/ clients at intermediate cardiac risk (i.e., stable angina),

and they should not be used in patients at higher risk (i.e., unstable angina) without clearance and planning with a physician. Vasoconstrictor use may also be contraindicated in patients/clients taking non-selective beta-blocker drugs (e.g., propranolol).

Smoking cessation should be encouraged

Myocardial infarction

Myocardial infarction (MI) results from a reduction in blood flow through one or more of the coronary arteries, resulting in infarct, or death, of myocardium (heart muscle).

Peak mortality following an MI occurs in the first year, primarily resulting from the increased electrical instability of the myocardium (heart muscle) after the infarction.

Inflammation may contribute to myocardial infarctions, strokes, and other cardiovascular events. Dental hygiene procedures such as scaling and root planing may release bacteria into the bloodstream, potentially increasing vascular inflammation and thereby increasing the risk of a vascular event. - Heart failure (HF): where a damaged heart can no longer pump sufficient blood to the body is a potential post-MI complication. Signs/symptoms include swelling of the ankles, as well as shortness of breath and coughing (due to fluid accumulation in the lungs).

Patients/clients who have experienced a myocardial infarction may experience depression, fear, and disturbances in body image, which are associated with changes in lifestyle (e.g., dietary restrictions, exercise, and maintaining low stress levels).

Oral manifestations

None are specific to myocardial infarction or coronary atherosclerotic heart disease; however, medications used in post-MI care and its complications can result in oral changes such as stomatitis (e.g., from antiarrhythmic use).

Side-effects of anti-hypertensive medications that may be used in post-MI patients/clients include:

Chronic cough (e.g., angiotensin converting enzyme inhibitors-ACEIs); taste changes (e.g., ACEIs, beta blockers, alpha-adrenergic blockers); angioedema of lips, face, tongue (ACEIs, angiotensin II receptor blockers-ARBs); upper respiratory tract infections (e.g., ARBs); gingival hyperplasia (e.g., calcium channel blockers-CCBs); xerostomia (e.g., thiazide diuretics

and alpha-adrenergic blockers); lichenoid reactions (e.g., thiazide diuretics and beta blockers); and lupus-like oral and skin lesions (e.g., direct vasodilators).

Dental management

Potential risks relevant to dental hygiene care post-MI include cardiac arrest, new myocardial infarction, angina pectoris, heart failure (HF), bleeding tendency secondary to antiplatelet/anticoagulant use and electrical interference with a unipolar pacemaker.

Typical post-MI drugs include antihypertensives (usually an angiotensin-converting enzyme inhibitor-ACEI), anticholesterol agents, and low dose ASA.

Medical complications in the dental hygiene setting can be minimized by booking short appointments and by terminating the appointment if the patient/ client becomes fatigued or short of breath, complains of chest pain, or develops change in pulse rhythm or rate; these signs and symptoms should prompt medical referral.

Because blood pressure is lowest in the afternoon, afternoon appointments may be preferred in post-MI patients/clients prone to hypertension. Alternatively, morning appointments may be preferred when the patient/client is well rested and has a greater physical reserve. The patient/client should be engaged to determine the best time of day for an appointment. A stress reduction protocol may be utilized where indicated.

General management strategies for patients/clients with a past history of MI without ischemic symptoms and no other risk factors include the following: short appointments in the morning, comfortable chair position (avoiding rapid position changes1), pre-treatment vital signs, and availability of nitroglycerin.

In addition to medical treatment, increasing numbers of persons experiencing MI undergo percutaneous coronary intervention (i.e., angioplasty + stent). To avoid stent thrombosis, patients/clients are typically placed on dual antiplatelet therapy (e.g., ASA and clopidogrel) following stent placement in the coronary arteries. For patients/clients who have received bare metal stents, dual antiplatelet therapy is typically continued for 6 weeks, after which clopidogrel may be discontinued. Elective surgery or procedures involving significant bleeding should be deferred until clopidogrel is discontinued. In contrast, in patients/clients receiving drug-coated (drug-eluting) stents, clopidogrel is usually continued for at least a year after

implantation; elective surgery or procedures involving significant bleeding should be delayed until after this one-year period. For both types of stents, ASA is generally continued for life.

If the dental hygienist is concerned about potential peri-procedural or post-procedural bleeding, the patient/client's cardiologist should be contacted regarding the patient/client's antiplatelet regimen and optimal patient management should be discussed before discontinuing the antiplatelet medications. Such medications should not be discontinued prematurely given their importance in minimizing the risk of stent thrombosis, and discontinuation of these agents before dental hygiene procedures usually is unnecessary. However, if there is a concern that excessive bleeding may occur, clopidogrel may have to be stopped several days before dental hygiene care that involves tissue manipulation; medical consult/clearance should be sought in this regard. If scaling procedures are planned for a patient/client on the anticoagulant warfarin, the patient/client's physician should be contacted to confirm that the PT ratio (prothrombin time) will be two times normal or less, or international normalized ratio (INR) is less than 3.0. If the patient/client is taking a novel oral anticoagulant (NOAC), medical consultation/clearance should be sought; it should be a physician (not a dentist) who recommends interruption of an NOAC for a dental/dental hygiene procedure (which is usually not indicated for dental hygiene procedures).

There is virtually no dental hygiene indication to discontinue aspirin (ASA) treatment in patients/clients with a history of MI. Other nonsteroidal anti-inflammatory drugs (NSAIDs), however, should generally be avoided in patients/clients whose cardiac history includes an MI.

Digitalis (e.g., digoxin), while less commonly used now than historically, is sometimes used as part of post-MI medical therapy to increase contractility of the heart, particularly if congestive heart failure is present. The dental hygienist should be alert to signs of digitalis toxicity in patients/clients (e.g., anorexia, nausea, vomiting, altered vision, neurologic abnormalities, and facial pain), which should prompt medical referral. If not detected and managed early, digitalis toxicity can lead to cardiac arrhythmias, which may be potentially life threatening.

Antiarrhythmic agents used post-MI (e.g., quinidine, procainamide) may result in nausea and vomiting, as well as hypotension. Oral ulcerations may indicate agranulocytosis.

Cardiac pacemakers may be implanted in patients/clients' post-MI who experience certain dysrhythmias (arrhythmias). These devices vary in their sensitivity to electrical interference. Newer bipolar models provide shielding against interference, and typically they do not require any special consideration during dental hygiene care. Older unipolar models are less protected from electrical interference and can be adversely affected by mechanized dental instruments. If in doubt regarding model safety, the dental hygienist should seek the advice of the patient/client's cardiologist prior to the use of mechanized devices. Nonelectrical alternatives to avoid functional interference (e.g., manual instruments) should be considered in patients/clients with unipolar pacemakers. Care should be taken in an open clinical setting where electrical dental equipment may be used for an adjacent patient/client.

Persons who have had an MI are at elevated risk of future adverse coronary events. Re-infarction or other emergency situations in the dental hygiene office need to be managed by emergency medical services (EMS). - Annual influenza immunization may be associated with some degree of protection from myocardial infarction. Thus, dental hygienists should encourage post-MI patients/clients to receive an annual flu shot, and dental hygienists themselves should undergo annual immunization to decrease disease transmission.

Smoking cessation should be encouraged if patient/client still smokes post-MI.

Infective endocarditis

Infective endocarditis (IE) is an infection of the endocardium induced most frequently by staphylococci or streptococci that lead to general or systemic symptoms of infection, embolic phenomena and endocardial vegetations. The pathogenesis of IE is mainly attributed to the formation of septic vegetations, which are fibrin-platelet complexes embedded with bacteria on heart valves. The persistent nature of biofilms can also induce inflammation and contribute directly to chronic bacteremia and thromboembolic events, which are serious complications associated with IE.

It is known that most of the procedures performed in the oral cavity (e.g. tooth extraction, apical surgery and root scaling) cause bacteremia, which may subsequently lead to IE

It has been reported that the percentage of endocarditis associated with dental procedures ranges from 4% to 14% of all cases. There has been a well-established practice of administering antibiotics to patients who are at risk of

developing IE prior dental treatment. The rationale for this is that a high circulating dose of antibiotics may prevent the development of infected vegetation on damaged endocardium.

Guidelines in many countries have recommended that before invasive dental procedures, antibiotics should be administered to people at high risk of IE. According to the guidelines from the American Heart Association (AHA), subjects with prosthetic cardiac valves, previous IE, unrepaired cyanotic congenital heart disease (CHD), completely repaired congenital heart defect with prosthetic material or device during the first six months after the procedure, repaired CHD with residual defects at the site or adjacent to the site of a prosthetic patch or prosthetic device, and cardiac transplantation recipients who develop cardiac valvulopathy are at risk of developing IE when they undergo an invasive dental procedure.

Table shows guidelines for IE prophylaxis in adults according to the AHA and to the British Society for Antimicrobial Therapy (BSAT) are shown (Gould et al, 2006; Wilson et al, 2007)

America heart association guidelines (2007)

Situation	Agent	Regimen: single dose 30 to 60 min before procedure
Oral	Amoxicillin	2g
Unable to take oral medication	Ampicillin	2g IM or IV
	Cefazolin or Ceftriaxone	1g IM or IV
Allergic to penicillin	Cephalexin *$	2g
	Clindamycin	600 mg
	Azithromycin or Clarithromycin	500 mg
Allergic to penicillin and unable to take oral medication	Cefazolin$ or Ceftriaxone	1g IM or IV
	Clindamycin	600 mg IM or IV

*or other first or second line oral cephalosporin in equivalent adult or pediatric dosage.
$ Cephalosporins should not be used in patients with immediate hypersensitivity reaction to penicillin (urticaria, angioedema or anaphylaxis).

British Society for antimicrobial therapy guidelines (2008)

Agent	Regimen
Amoxicillin	3 g 1 h before procedure
Amoxicillin	1 g IV just before procedure or at time of induction of anesthesia
Clindamycin	600 mg 1 h before procedure

Clindamycin	300 mg IV given over at least 10 min
Azithromycin	500 mg oral suspension for patients that cannot swallow capsules 1 h before procedure

Dental management of infective endocarditis

Management of patients with infective endocarditis will involve Health questioning which will cover history for all potential categories of risk. If any doubt exists, the patient's physician should be consulted. Oral hygiene should be practiced with methods that improve gingival health yet minimize bacteremia. In patients with significant gingival inflammation, oral hygiene is initially limited to gentle procedures. Oral irrigators are generally not recommended because their use may induce bacteremia. Susceptible patients should be encouraged to maintain the highest level of oral hygiene once soft tissue inflammation is controlled.

Severe periodontal disease and areas of periodontal suppuration or dental focus of infection require elimination. Pretreatment chlorhexidine mouth rinses are recommended before all procedures, including periodontal probing, because they significantly reduce the presence of bacteria on mucosal surfaces. Dental extraction should be avoided in healthy mouths whenever possible. Endodontic therapy is the treatment of choice. Also, single extractions are preferable to multiple extractions. All dental treatment procedures require antibiotic prophylaxis. When possible, at least 7 days are kept between appointments (preferably 10-14 days). If this is not possible, an alternative antibiotic regimen is selected for appointments within a 7-day time period. Regular recall appointments, with an emphasis on oral hygiene reinforcement and maintenance of oral health, are extremely important for patients with infective endocarditis.

Dental management of dysrhythmia patients

A specific diagnosis of an arrhythmia during a dental appointment necessitates continuous ECG monitoring and good knowledge of interpretation of the abnormalities observed. Thus, in the usual dental setting, patient history, symptoms, and palpation of the pulse are the available diagnostic tools. The risk of harmful arrhythmias is also increased in patients with cardiomyopathies, heart failure, and valvular problems. Such patients should be carefully evaluated by their physician and adequate medication and other measures (such as an implantable cardioverter-defibrillator) should be implemented before extensive dental procedures. If a patient with known heart disease develops arrhythmia during treatment, the treatment should be discontinued, supplemental oxygen considered, and the patient status closely

monitored. If the patient recovers quickly, continuation of treatment may be considered if the patient wishes. Even a brief loss of consciousness, however, may indicate significant cardiac arrhythmia, and the patient should be referred to medical evaluation. If a patient with heart disease collapses in the chair, cardiac arrest should be suspected and emergency medical services activated immediately and cardiopulmonary resuscitation initiated without delay. These patients are advised to take their medication regularly. Beta-blockers are the preferred drug of choice.

Dental treatment of patients with anticoagulant therapy

Patients on anticoagulant therapy should be delicately handled in a dental setup. This may involve use of local hemostatic measures to control bleeding in anticoagulated patients. These include atraumatic surgical technique, adequate wound closure, pressure application, and topical clotting agents. Oral rinsing with tranexamic acid can also be used. The indication for anticoagulation should be known since many indications allow brief discontinuation of anticoagulant treatment without a substantial increase in the risk of thrombotic events. On the other hand, anticoagulant treatment should in general not be discontinued in patients with mechanical valve prostheses. Close collaboration with the patient's physician is recommended in these matters. In patients receiving long-term anticoagulant therapy and who are stably anticoagulated on warfarin, an international normalized ratio (INR) check 72 h prior to surgery is recommended. This allows sufficient time for dose modification if necessary to ensure a safe INR (2-4) on the day of dental surgery (including subgingival scaling). There is no need to check the INR for non-invasive dental procedures.

Pain is usually referred to the jaw in case of heart attack. One of the first symptoms of a heart attack can be pain in the teeth and/or jaws. In the case of a heart attack, the presence of pain in the teeth or jaws doesn't mean that there is any dental condition that needs to be treated, in the absence of any pre-existing dental condition. The experience of dentally related pain during a heart attack is a classic example of referred pain which is pain felt at a site distant from the site of origin. But referred pain can also occur under less dramatic circumstances unrelated to any cardiac pathology. Thus, pain referral is frequently found in patients with chronic musculoskeletal pain (for example, temporomandibular disorder (TMD), fibromyalgia, and chronic low back pain). In patients with TMD, for example, muscle and/or jaw joint pain could refer to the teeth and other parts of the orofacial area. Patients and clinicians alike can become convinced that the pain is actually due to some form of dental pathology and there are clinical cases where tooth extractions have been

carried out in the mistaken belief that there is a dental cause to the pain. In these circumstances, tooth extractions have no effect whatsoever on the cardiac pain.

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Chapter - 9
A Rare Disorder: Papillon-Lefèvre Syndrome and its Oral Features

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Chapter - 9

A Rare Disorder: Papillon-Lefèvre Syndrome and its Oral Features

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Abstract

Papillon-Lefèvre Syndrome (PLS) is a rare autosomal recessive disorder characterized by severe dermatological and dental symptoms, including palmoplantar hyperkeratosis and aggressive periodontitis. This chapter focuses on the oral features and dental therapy for PLS patients. The hallmark symptoms of PLS include severe gingivostomatitis, aggressive periodontitis, and premature loss of both deciduous and permanent teeth. The oral symptoms have profound implications for the patient's oral health and overall well-being, affecting their ability to chew, speak, and their social and psychological well-being. Therapeutic options for primary dentition are limited, and many individuals with PLS become edentulous by their early teens. Research suggests that PLS patients may have compromised immunological or inflammatory defense systems, with a primary focus on polymorphonuclear neutrophil leukocytes (PMNs). Deficiencies in PMN function have been observed in PLS patients, and PMN dysfunction may be associated with a toxic impact from extracellular toxins and lipopolysaccharide antigens from periopathogens. Successful conservative periodontal therapy in a PLS patient has resulted in normalizing PMN and monocyte functions and the elimination of Actinobacillus actinomycetemcomitan. This chapter aims to provide insights into the etiology of periodontal disease in PLS and encourage healthcare professionals to develop treatment plans to preserve permanent teeth and alveolar bone.

Keywords: Papillon-lefèvre syndrome, periodontitis, palmoplantar hyperkeratosis, premature tooth loss, autosomal recessive disorder, gingivostomatitis

1. Introduction

Papillon-Lefèvre Syndrome (PLS) is a rare autosomal recessive disorder that presents a unique set of challenges for those affected. It is primarily

characterized by severe dermatological and dental symptoms. Furthermore, PLS is characterized by extensive palmoplantar hyperkeratosis and severe periodontal inflammation, leading to premature loss of both primary and permanent teeth. PLS belongs to a group of skin disorders referred to as palmoplantar keratodermas, which are genetically and clinically diverse [1].

All groups of skin disorders are identifiable by hyperkeratotic lesions on the palms of the hands and soles of the feet. PLS is distinguished from other palmoplantar keratoderma (PPK) disorders by the early and severe onset of periodontitis. Haneke's classification of PLS is based on specific criteria, including palmoplantar hyperkeratosis, loss of primary and permanent teeth, and autosomal recessive inheritance [1].

The diagnosis of PLS involves a clinical examination of the skin and oral tissues. Additionally, the mode of inheritance can be determined through a pedigree, while mutation analysis can detect mutations in blood or tissue samples [2].

Inheritance of PLS follows an autosomal recessive pattern. Individuals born to parents, both carrying the faulty gene, have a 25% chance of developing the illness. Heterozygous carriers of the PLS gene, accounting for approximately 2-4 per 1,000 individuals, contribute to a population prevalence of one case in every 1-4 million persons. Notably, the incidence of PLS is higher in isolated societies, particularly in the presence of consanguineous marriages, responsible for about one-third of all PLS cases [3].

Approximately 20% of individuals affected by PLS are more susceptible to infections, along with periodontal disease. Common medical issues among PLS patients include painful fissures and recurrent pyogenic skin infections, with reported cases of liver abscesses or pseudotumors. Additionally, growth retardation, non-symptomatic intracranial calcifications, and mental retardation may be observed in PLS individuals [4].

Studies have linked PLS disorder to severe periodontal disease, leading to premature tooth and bone loss in young patients. The associated oral symptoms can pose functional and cosmetic challenges, affecting the psychological and social well-being of affected children. Maintaining proper oral health significantly improves the quality of life for individuals living with PLS [4].

This chapter aims to provide the latest insights into the etiology of periodontal disease in PLS and encourage healthcare professionals to develop treatment plans to preserve permanent teeth and alveolar bone. Numerous investigations suggest that patients with PLS may present distinct

characteristics. Therefore, this review article focuses on dental therapy for PLS patients while excluding case reports unrelated to oral health concerns.

2. Oral features of PLS

The condition known as PLS is primarily characterized by severe periodontal disease, leading to early and significant tooth loss. In young patients with PLS, oral mucosal and gingival tissues initially appear normal, but following the eruption of primary teeth, the gingival tissues become inflamed, swollen, and susceptible to bleeding. These changes are followed by rapid deterioration of the periodontal tissues, resulting in increased tooth mobility [5, 6].

2.1 Severe gingivostomatitis

One of the hallmark symptoms of PLS is severe gingivostomatitis. This condition involves inflammation and ulceration of the gums and oral mucosa, leading to discomfort and pain. Patients often experience redness, swelling, and bleeding of the gums, which significantly impacts their oral health and overall quality of life [7].

2.2 Aggressive periodontitis

PLS is closely associated with aggressive periodontitis, a rapid and severe form of gum disease. This condition leads to the destruction of the periodontal ligament and alveolar bone, causing teeth to become loose and eventually fall out. The aggressive nature of the periodontitis in PLS patients means that both deciduous (baby) and permanent teeth are often lost prematurely [8].

2.3 Eruption and Exfoliation of teeth

Children with PLS typically experience the eruption of their deciduous teeth at the expected age. However, due to the severe periodontitis, these teeth are often lost by the age of five. As the permanent teeth erupt, they too are affected by periodontitis and are usually lost by the age of 15, leading to significant dental and functional challenges for the patient [9].

3. Impact on oral health

The oral symptoms of PLS have profound implications for the patient's oral health and overall well-being. The premature loss of teeth affects not only the ability to chew and speak but also has social and psychological impacts, particularly in young patients. The ongoing inflammation and infection require continuous dental care and often involve multidisciplinary approaches, including dermatologists and periodontists [10].

Therapeutic options for primary dentition are limited due to the young patients' inability to engage in oral hygiene assessments and conservative periodontal therapy, such as scaling and professional teeth cleaning. Consequently, the majority of primary teeth in PLS patients develop significant periodontal disease, leading to premature shedding at a young age. Some children with this condition may require primary tooth extractions to address severe periodontal issues, after which the gingival tissues undergo a restorative process [11].

Following the eruption of permanent teeth, the inflammatory process may recur, causing further periodontal damage. The severity of periodontal disease varies between primary and permanent dentitions, but conservative therapy is often efficacious. Nevertheless, many individuals with PLS become edentulous by their early teens [12].

It has been observed that patients with PLS may have compromised immunological or inflammatory defense systems, as evidenced by severe periodontitis and increased susceptibility to infections. Despite being rare, the periodontal inflammation observed in PLS patients has spurred extensive research into cell-mediated immune responses, with a primary focus on polymorphonuclear neutrophil leukocytes (PMNs). PMNs play a crucial role in the immunological response to bacterial plaque near the gingival margin, where their functions include attachment to the capillary endothelium, migration, chemotaxis, phagocytosis and bacterial eradication through oxidative and non-oxidative mechanisms [13].

Deficiencies in PMN function, including decreased chemotaxis, reduced and impaired lytic activity, poor leukocyte adhesion, and impaired generation or increased release of superoxide radicals, have been observed in PLS patients. Various investigative strategies are being explored to elucidate the inconsistent findings regarding PMN function. Additionally, PMN dysfunction may be associated with a toxic impact resulting from extracellular toxins and lipopolysaccharide antigens from periopathogens, potentially stemming from infection [14].

Successful conservative periodontal therapy in a PLS patient has resulted in normalizing PMN and monocyte functions and the elimination of Actinobacillus actinomycetemcomitans (A.a), further supporting the link between PMN dysfunction and infection. Furthermore, abnormalities in lymphocyte transformation and elevated levels of natural killer cells have also been reported in PLS patients, shedding light on the multifaceted immunological aspects of this syndrome [15].

Periodontal disease is linked to bacterial infection, with specific gram-negative bacteria commonly detected in lesions. However, no single bacterium has been linked to periodontitis. Periodontal disorders impact youngsters, with Actinobacillus actinomycetemcomitans (A.a) being the most often related pathogen, especially in PLS patients [16].

Antibody research indicates that infection with a periodontal pathogen increases serum IgG1, IgG2, and IgG3 titres against A.a. in young PLS patients. Other researchers have not found a relationship between PLS periodontitis and A.a. Other periopathogens, including herpes viruses, may also have a role in the development and progression of aggressive periodontitis [17].

4. The genetic factor of PLS

Two study groups identified the genetic cause of PLS as a lack-of-function mutation in the cathepsin C gene, thanks to advances in human genome research. Cathepsin C is a lysosomal cysteine proteinase present in several cell types, with a higher abundance in immune cells. It coordinates protein breakdown and activates serine proteases, which play important roles in immunological and inflammatory cells [18].

Over 41 cathepsin C gene variants have been found in PLS patients, all of which are homozygous. Compound heterozygous mutations in patients with PLS and "symptomless mutations" in the cathepsin C gene in homozygous people have been reported. Heterozygous carriers of the gene are not clinically impacted, albeit one heterozygous patient had plantar hyperkeratosis without periodontal disease [18].

Mutations in the cathepsin C gene have been confirmed in Haim-Munk syndrome. This disorder is comparable to PLS but also includes arachnodactyly, nail atrophic alterations, and hand phalange deformity. PLS and Haim-Munk syndrome are distinguished by the presence of severe early-onset periodontitis and palmoplantar keratoses, making it a unique diagnosis [18].

Some families with PLS exhibit both skin and periodontal lesions, while others only have palmoplantar keratosis and no periodontal tissue involvement in the primary or permanent dentition. The research included a family of six children, four of whom had palmoplantar lesions and two of whom had periodontal infection affecting solely their permanent dentition [18].

Another study found that individuals with palmoplantar keratoses experienced peridontal inflammation in the permanent dentition but not in the

primary dentition. Ullbro *et al*. studied 47 individuals with PLS and found no patients without periodontal disease in the primary dentition. PLS and other single gene diseases have diverse expressions that can be influenced by environmental circumstances. PLS patients often exhibit a wide range of phenotypes. It's unclear whether late-onset periodontal disease is caused by genetic diversity or if patients have a confirmed PLS mutation [18].

Mutations in the cathepsin C gene have also been documented in individuals diagnosed with prepubertal periodontitis. This condition showcases a periodontal state akin to PLS periodontitis, but without the accompanying dermatological indications. Some theories suggest that prepubertal aggressive periodontitis might be a version of PLS with varying genes in certain families, which could manifest as a partially penetrant PLS. Despite this, cases of aggressive periodontal disease aren't always linked to cathepsin C mutations. There isn't concrete proof that individuals bearing a low-activity cathepsin C variant will invariably suffer from aggressive periodontitis [18].

Though the genetic basis of PLS has been identified, the precise biological mechanisms behind the dermatological and periodontal lesions remain elusive. The cathepsin C gene is active in epithelial regions typically impacted by PLS like the palms, soles, knees, and keratinized oral gingiva. However, the specific ways in which mutations in the cathepsin C gene lead to or play a role in PPK are not thoroughly understood as its functions in the epidermis haven't been extensively explored. Some postulations propose that cathepsin C plays a crucial role in establishing and maintaining the structural organization of the epidermis in extremities, as well as the integrity and immunological characteristics of tissues surrounding the teeth. It might indirectly aid in protein processing like keratins, crucial for maintaining the structural integrity of epithelia unique to PLS-affected tissues [18].

Regarding the defence mechanisms of the organism, studies on cell lines from cathepsin C-deficient mice reveal that cathepsin C is key for activating serine proteinases in immune and inflammatory cells. This activation is vital for granule-mediated apoptosis of tumor cells and infected cells by cytotoxic lymphocytes. Based on observations from cathepsin C-deficient mice, individuals with PLS might exhibit a general immunodeficiency due to the lack of protesase activation. However, investigations on PLS patients have not indicated a widespread T cell immunodeficiency phenotype, nor do these patients appear to be exceptionally prone to viral infections [18].

Studies have shown that patients with PLS maintain significant granzyme activities in cytotoxic lymphocyte compartment, showcasing well-preserved cytotoxic lymphocyte function and normal LAK cytotoxicity against K562 cells. Nevertheless, inactive granzyme B in resting natural killer cells of humans with PLS suggests that cathepsin C is crucial for activating granzyme B in unstimulated human NK cells. This cytolytic defect in NK cells was consistently and severely observed in all PLS patients in various studies [18].

Human keratinocytes in culture release granzyme B, which has antibacterial properties and protects against infections. The activation of granzyme B may thus give protection against skin irritation. Failure to activate granzyme B in PLS may decrease the epithelial response to bacterial infection, leading to gingival inflammation [18].

A lack-of-function mutation of the cathepsin C gene may contribute to PMN dysfunction, as previously documented. Cathepsin C activates serine proteinases such as cathepsin G, elastase, and proteinase 3 in PMNs, which are crucial for phagocytic bacteria elimination. Two brothers with PLS showed virtually total loss of cathepsin G and elastase activity. Loss of cathepsin C activity leads to decreased activity and stability of neutrophil-derived serine proteases. However, a study found that neutrophils from patients with PLS do not consistently have a defect in their ability to kill Staphylococcus aureus and Escherichia coli [18].

The authors proposed that serine proteases are not the primary method by which human neutrophils kill common bacteria. Instead, other systems play a role.

Serine proteases play a crucial role in activating and deactivating cytokines on a local level. In addition, there are various inflammatory mediators that cause extracellular matrix breakdown [18].

5. Treatment and Management

Managing the oral symptoms of PLS is challenging and requires a comprehensive approach. Treatment strategies often include rigorous oral hygiene practices, regular dental check-ups, and sometimes surgical interventions to manage periodontal disease. Antibiotic therapy is also commonly used to control infection and inflammation. Early and aggressive treatment is crucial to mitigate the severe oral manifestations of this syndrome and improve the patient's quality of life [19].

There is significant variation in the oral symptoms of PLS patients. Previous studies have reported on the effects of different oral treatment

regimens, but the impact of heterogeneity on treatment outcomes has been overlooked. Many studies have limited patient populations, making it impossible to address this factor [19].

Early considerations. The therapy for PLS periodontitis generally involved extraction of badly damaged teeth. Later attempts using mechanical scaling and chemotherapy were unsuccessful. To provide an infection-free interval before permanent teeth develop, all primary teeth were pulled at an early age and treated with systemic tetracycline. The excision of all primary teeth was recommended due to significant inflammation in the primary dentition. Others have successfully adopted this therapeutic strategy [19].

Antibiotics targeting A.a, a gram-negative periopathogenic bacterium, may be effective in treating PLS periodontitis. Previous research has shown both successful and failed systemic usage of antibiotics such erythromycin, penicillin, amoxicillin, clavulanic acid, and tetracycline. Two individuals with PLS successfully received long-term systemic tetracycline therapy. Tetracycline, including low-dose doxycycline, minocycline, and tetracycline HCL, may reduce collagenase synthesis, irrespective of their antibacterial effects [19].

Tetracycline isn't recommended for kids under 12. Instead, a combination of amoxicillin and metronidazole has been proven effective against A.a. in treating PLS periodontitis. This treatment, taken for seven days, has shown to clear A.a. for up to two years post-treatment in those with chronic periodontitis and PLS [19].

In a recent study, a PLS patient treated with amoxicillin/metronidazole showed improvement despite recurrent A.a. findings, suggesting the involvement of other microbes [19].

Additional treatments for PLS patients include using chlorhexidine solutions for rinsing or subgingival irrigation and regular professional cleanings like scaling and root planning. Some studies suggest that synthetic retinoids help improve periodontal health, while others argue their ineffectiveness [19].

Many patients with less severe periodontal disease can retain most of their teeth into adulthood. A systematic treatment approach involving regular follow-ups every three months, dental hygiene education, professional cleanings, and timely periodontal interventions has shown success in young PLS patients. Early extraction of primary teeth before permanent teeth eruption has led to fewer permanent teeth loss and reduced signs of periodontal disease compared to those treated later in life [19].

Maintaining good oral hygiene was crucial for the success of the treatment protocol. Sometimes, antibiotics had to accompany conservative periodontal treatment to address any instances of gum inflammation. Extracting primary teeth before permanent molars erupt might play a significant role in preserving the final set of teeth. It's possible that younger patients could face gum diseases later in life, like cases of delayed periodontitis in individuals with PLS. However, a study revealed that most PLS patients who developed gum disease showed symptoms before nine years of age. Interestingly, PLS individuals are less likely to experience gum issues once they pass their teenage years [19].

For individuals with PLS and severe tooth loss, functional and cosmetic challenges are common. Many have found success in restoring their smiles through prosthetic solutions like osseointegrated implants. With the premature loss of primary teeth, it's not unusual to see adult teeth like premolars and canines out of position. Following effective gum therapy, there might be opportunities for controlled tooth movements to address these concerns [19].

However, a study revealed that most PLS patients who developed gum disease showed symptoms before nine years of age. Interestingly, PLS individuals are less likely to experience gum issues once they pass their teenage years [19].

6. Conclusion

In conclusion, the oral symptoms of Papillon-Lefèvre Syndrome are a defining and distressing component of the disorder. Understanding and addressing these symptoms through diligent care and a multidisciplinary approach is essential in managing this rare condition.

The phenotypic expression of Papillon-Lefèvre syndrome (PLS) exhibits a wide range of severity in both dermatological and periodontal symptoms. This rare syndrome is caused by mutations in the gene responsible for cathepsin C, which leads to a loss of function of this lysosomal cysteine proteinase. As a result, there is a significant decrease in the levels and activity of neutrophil-derived serine proteases and in the activation of granzyme B within natural killer cells. While most individuals with PLS do not typically suffer from severe infections, the oral cavity presents a specific vulnerability due to the prevalence of aerobic and anaerobic pathogens, which may pose a risk to a potentially weakened innate immune response.

Research indicates that consistent reduction of periopathogen flora in the oral cavity and early initiation of effective antibiotic therapy at the first sign of any potential infection have shown that individuals with PLS may not

inevitably experience complete tooth loss in their permanent dentition. This underscores the importance of proactive periodontal care and regular monitoring to manage the unique oral health challenges associated with PLS.

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Preface

The 5th International Conference on Advances in Information Communication Technology and Computing (AICTC 2024) was held at Zhanibekov University, Shymkent, Kazakhstan, from April 29–30, 2024. The aim of AICTC 2024 was to present ongoing research in the field, foster research relations between universities and industry, and provide participants with a review of the latest and upcoming trends. This conference offered a leading-edge scholarly forum for researchers, engineers, and students to share their state-of-the-art research and developmental work in the broad areas of pervasive computing and communications.

The conference received around 317 research papers from various parts of the world, including submissions from countries like Russia, Oman, Ghana, the USA, Kazakhstan, Iraq, Malaysia, Indonesia, Japan, and others. These papers were reviewed by experts in the field, and based on the reviewers' comments and decisions, 50 papers were accepted for presentation at the conference. AICTC 2024 extends its gratitude to the reviewers for their time and suggestions in selecting the research papers for the conference.

The selected papers were presented by their respective authors during the conference sessions. Session chairs and the audience discussed the methodology and results of each paper in detail, incorporating suggestions to enhance their usefulness. A total of 12 sessions were held, in addition to the inaugural and valedictory functions. Each session featured a keynote speaker whose talk was related to the session's theme, enriching the event with knowledge and innovation.

The selected and presented papers at the conference are submitted to Springer for publication in the reputed LNNS series. The Hari Krishan Research and Development Nexus provided financial support for the proper organization of the conference. We express our thanks to the speakers: Dr. Aninda Bose, Executive Editor at Springer Nature Group, London, UK; Prof. Prashant Jamwal, Nazarbayev University, Kazakhstan; Tomas Cech, Institute of Education and Social Science, Czech Republic; Dr. Amit Joshi, Director GR Scholastic LLP and Knowledge Chamber of Commerce and Industry of India; Dr. Ketan Kotecha, Director, Symbiosis Institute of Technology, SIU, India; Dr. Arvind Chalasali, Jakarta, Indonesia; Dr. Manju

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We are grateful to the delegates and authors for their participation and interest in AICTC 2024 as a platform to share their ideas and innovations. Special thanks to Dr. Gulzhan Sugirbayeva, Rector, and Dr. Yerzhan Kerimbekov, Vice-Rector, Zhanibekov University, Kazakhstan; Dr. Aditi Sharma, Department of Computer Science and Engineering, Symbiosis Institute of Technology, Symbiosis International (Deemed University), Pune, India; Dr. Padma Joshi, Jain Girls PG College, Bikaner; Deepak Acharya, Jain Girls PG College, Bikaner; Dr. Jyoti Gajrani, Engineering College, Ajmer; and Dr. Rituraj Soni, Engineering College, Bikaner. We extend our heartfelt gratitude to the reviewers and technical program committee members for their efforts during the review process. We are thankful to everyone directly or indirectly associated with the conference organizing team for their contributions to its success.

We hope you enjoy the conference proceedings and wish you all the best.

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Deep Learning for Identification of Dental Implant Systems Using Radiographic Images—A Pilot Study

Veena Benakatti, Ramesh P. Nayakar, and Mallikarjun Anandhalli

Abstract Dental implants are a well-accepted form of prosthetic alternative for missing teeth. Owing to their demand, over 220 manufacturers have entered the market and produced a variety of implants with different make and shape. This creates a challenge for dental practitioners to identify the implant brand when the necessity arises. There is no well-established method for implant identification, and this calls for a quick and scientific method. Deep learning, a computer vision technique for image recognition, can be a solution, to identify implants in a radiographic image. The objective of the study was to evaluate the efficacy of deep learning in implant identification using radiographs. A dataset of 740 images consisting of Osstem TSIII SA, Dentium superline, and Adin Touareg implant systems was derived from panoramic and periapical radiographs to train the YOLOv8 model in implant identification. The model identified Adin Touareg with 94% accuracy, Osstem TSIII SA with 90% and Dentium superline with 85% accuracy giving an overall accuracy over 90%. In the current study, we found that the YOLOv8 model could accurately identify implants in radiographs even with smaller training datasets, and more cross-sectional studies across the world with diverse implant systems and a larger sample size are recommended for generalizability and to be able to implement the model in a real-time scenario.

Keywords Artificial intelligence · Deep learning · Dental implant systems

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1 Introduction

Dental implants are one of the well-accepted prosthetic alternatives for missing teeth. Owing to their popularity, over 220 implant brands have entered the market and variety continues to grow. The make and shape of the dental implants are unique to the company. After placement implants will need maintenance and repair in due course of time due to biologic and mechanical complications, during this phase information about the implant system is required [1]. Identifying the system and procuring tools or components specific to the company is necessary during this phase. Currently, patient previous records and radiographs are the only tools in identifying the system. This becomes challenging to clinicians when patients do not have or lost implant records or traveled from other regions or countries. Clinician tries to make an assumption about the type of system looking at implant make and shape in a radiographic image which requires a significant amount of human effort and experience. Clinicians either rely on radiographic interpretation of the implant system or contact the company or their colleagues to seek help in identifying the system. In either case, implant identification becomes an assumptive process not a definite approach. Until today there is no well-established method of implant identification. Several attempts have been made to document the basic design and features of implant systems on radiographs [2–5]; however, none of them are easy or quick and require a significant amount of human effort, knowledge, experience, and time. Currently, artificial intelligence (AI) is gaining importance in medical imaging diagnosis owing to its ability to analyze complex patterns in the image and provide classification and predictions. Machine learning (ML) and deep learning (DL) a subset of AI can be adapted to analyze the image of an implant in a radiograph and classify or predict the type of implant system.

Artificial intelligence, a branch of computer science, can replicate human cognition to produce human-like responses to accomplish specific tasks. Machine learning, a subset of artificial intelligence works on algorithms and constitutes statistical models which enable computers to learn from data and improve their performance through experience without being explicitly programmed [6]. A special class of ML suited for image classification is deep learning. DL is an evolution of ML that automates data preprocessing to generate features to optimize classification tasks. Considering the utility of ML and DL, they have been applied for image-based diagnosis in dentistry. ML and DL can be used to analyze, interpret, classify, and predict from radiographs, photographs, and medical data for assessment and diagnostic purposes [7].

2 Literature Survey

Dental implants have been in practice for a long time and have proven to remain the future of dentistry. As long as implants remain in patients' oral cavities, they will need maintenance and repair, implant identification is an indispensable part of implant maintenance. There is a continuous thrust in recognizing a method for implant identification. Sahiwal et al. [2, 3] documented the basic design and identifying features of implants, thereby helping dentists to recognize implants from radiographs. This requires the clinician to read the features of the implant in the radiograph and guess the implant system. Michelinakis et al. [4] developed implant recognition software that identified the unknown implant using a range of criteria. However, the database consists of the implant characteristics derived from the specifications given by the manufacturer in the brochure. Several leading questions regarding the implant in question will reduce the number of possibilities and provide matching implants based on inputs, and then the dentist has to match with that of the patient's implant. This requires the dentist to verify if both images of the implant (implant image in question and implant image from database) are matching to identify the implant in question. Conversely, in the current study, the AI model itself recognizes the implant based on a radiograph. Rami Jandali [5] devised a radiofrequency chip loaded with implant information, which can be fitted into an implant screw, and a wireless reader that can communicate to the chip and retrieve the information. The wireless reader emits electromagnetic waves to activate the chip which could pose a hazard to human health and all clinicians may not have the specialized device at the clinic. Currently, radiographs are the only means by which clinician interprets information to predict the type of implant system. Considering the rapid growth of implant dentistry there is a necessity for a quick and appropriate scientific method for implant system identification.

A few studies [1, 8–11] later from 2020 onwards used deep learning and convolutional neural networks for the recognition and classification of dental implants in panoramic and periapical radiographs. This technique uses radiographic images to train DL algorithms to perform specific tasks such as identifying implants. These studies reported that AI techniques achieve near-perfect accuracy in identifying different dental implant systems. These studies presented some important insights such as the models were able to identify implants with distinct features more proficiently and the larger the parameters and deeper the network was, the better the performance of the models. However, some studies considered data augmentation which may lead to data bias, as the copied images are quite different from original leading to suboptimal performance.

Cross-sectional studies across the world including diverse implant systems, algorithms, and different radiographic devices for image capturing will help build a robust algorithm that can be applied in a real-time scenario.

3 Methodology

3.1 Ethical Approval

The study was approved by the university ethical committee (KAHER/EC/21-22/D-290721002). The study used anonymized intraoral implant radiographs for training the DL model and did not include any other patient data. Since the study was of retrospective non-interventional design, individual informed consent was waived off from the committee.

3.2 Study Design

The objective of the study was to evaluate if deep learning can identify dental implant systems in radiographs. Deep learning was implemented using the convolutional neural networks (CNN) model you only look once (YOLOv8).

3.3 Data Collection

A dataset of digital periapical and panoramic radiographs of implants was obtained from dental institutions and practitioners across India. Radiographs were kept anonymized to maintain the confidentiality of the records. The radiographs were screened by an oral radiologist, and only standard-quality images were included for the study. Radiographs with unknown implants, distortion, haziness, blur, and other conditions that impede the identification of implant systems were excluded. Images were exported as JPEG images. Osstem TSIII SA, Dentium superline, and Adin Touareg implant systems were used to train the DL model (Fig. 1). A total of 740 images were extracted from periapical and panoramic radiographs. Implant images at various treatment stages such as implants with healing abutments, temporaries, and final prostheses were included.

3.4 Choosing an AI Model

CNN, a deep learning technique, is preferred for computer vision tasks such as image segmentation and image classification. YOLOv8 model, the fastest and most state-of-the-art object detection algorithm that has become a standard way of detecting objects in computer vision was trained to detect implants. YOLOv8 is a one-stage algorithm that has high real-time performance and precision in multi-scale object detection. YOLOv8 is the latest iteration of the YOLO series building on the older

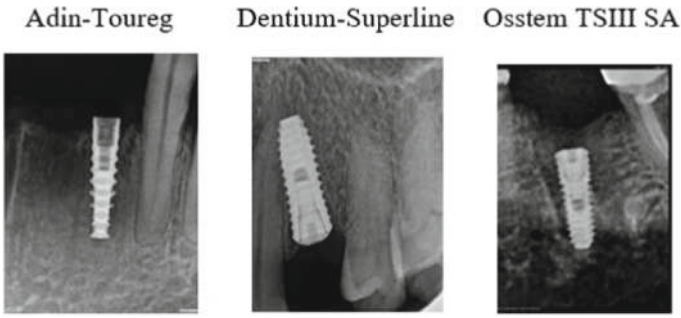


Fig. 1 Sample data

version that has new features and optimizations making it ideal for object detection [12].

3.5 Training of Model

Image classification involves data collection, labeling, training, and performance metrics. Figure 2 gives the schematic illustration of the steps involved in training the YOLOv8 model. Once the data was collected, types of implant systems and the corresponding images were categorized and labeled using patient records from the department ledger. Images were divided into two datasets: one for training (80%) and another for testing (20%). The training dataset was used to train the model by learning, and the testing dataset that was independent of the training dataset was used to analyze the model's performance. Annotation of the data included bounding box coordinates and class labels (Fig. 3). The training was carried out on YOLOv8, and the progress was monitored to fine-tune parameters. The model was initially trained for 50 epochs without resizing the input images as the model performed well without this preprocessing step, focusing on the detection of three specific classes, and no augmentation was applied during training.

3.6 Testing of the Model

The trained model was analyzed for its performance using the test dataset and performance metrics; confusion matrix, precision, and recall rates were recorded and tested.

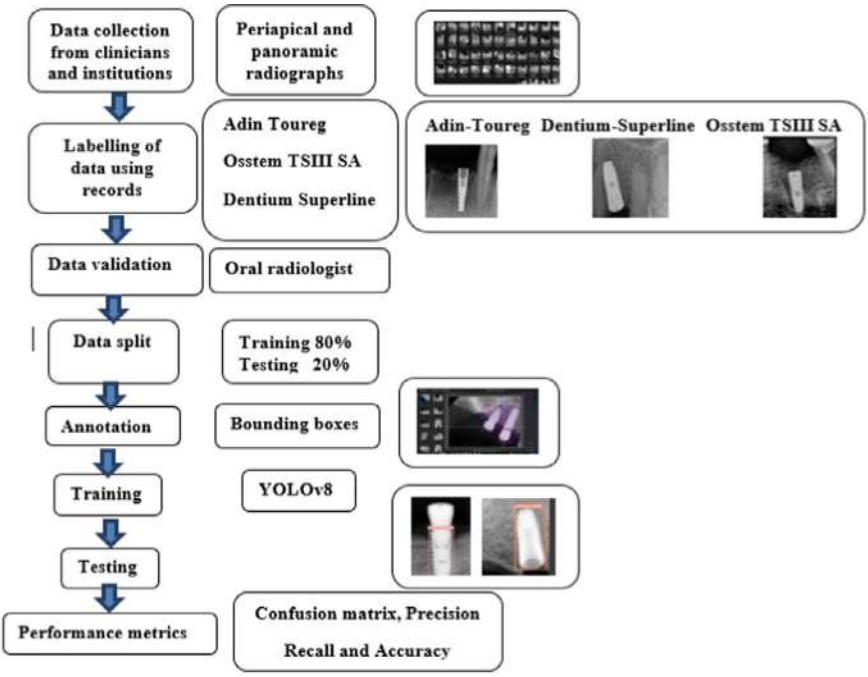


Fig. 2 Schematic illustration of methodology

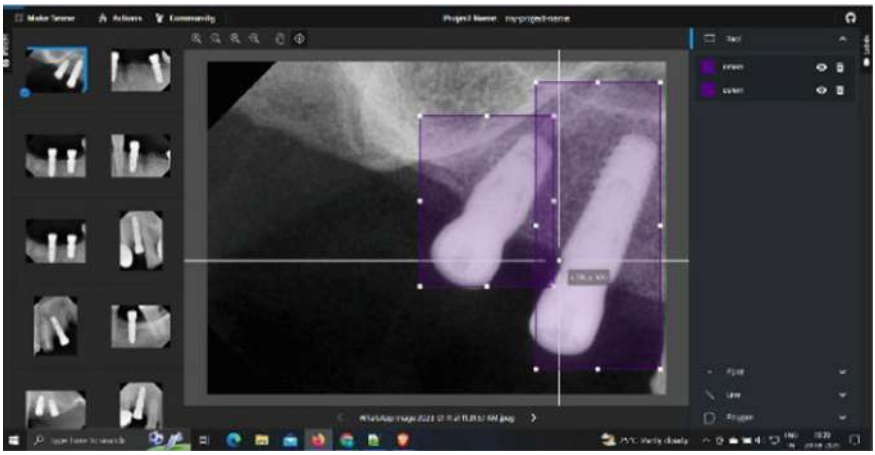


Fig. 3 Data annotation

4 Results

The performance matrix of the YOLOv8 model is represented in Figs. 4 and 5. Figure 4 shows the normalized confusion matrix, and Fig. 5 represents the training graphs of YOLOv8.

The multi-class confusion matrix (Fig. 4) represents the performance of the YOLOv8 model and indicates model confusion between the classes, and the implant systems in the present case. It shows the extent to which it is accurately recognizing a particular implant system and instances where it is getting confused with other implant systems. The diagonal line represents the accurately predicted implant systems giving an overall accuracy over 90%. The model identified Adin Touareg with 94% accuracy, the highest among all three systems. The next best accuracy of 90% was found with the Osstem TSIII SA followed by the Dentium superline at 85%.

Figure 5 represents the training and performance graphs generated for the YOLOv8 model. The horizontal axis shows the number of epochs and the vertical

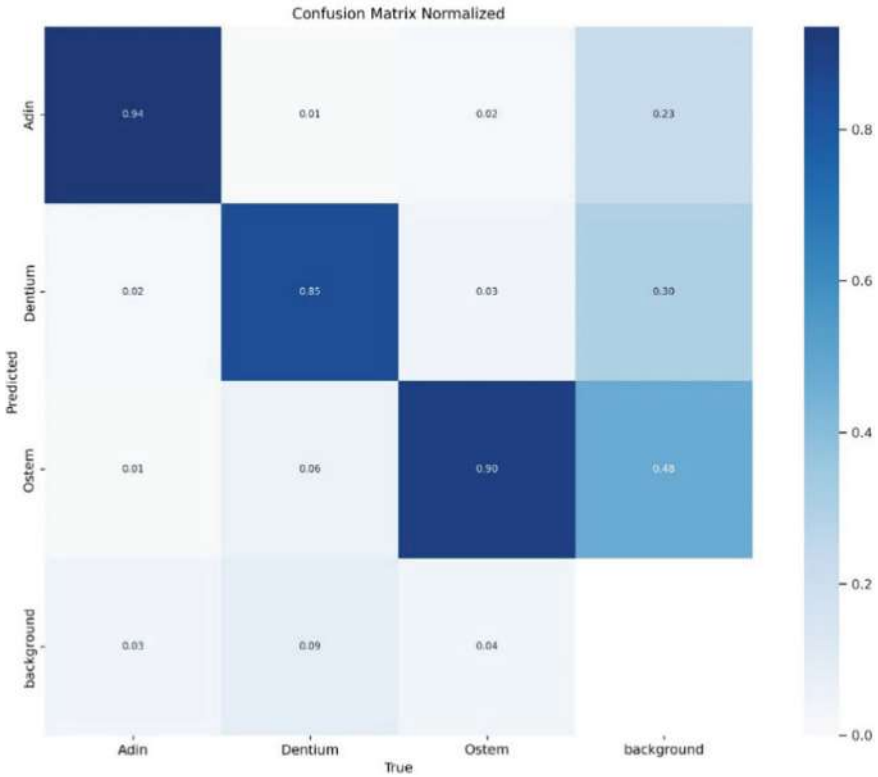


Fig. 4 Normalized confusion matrix

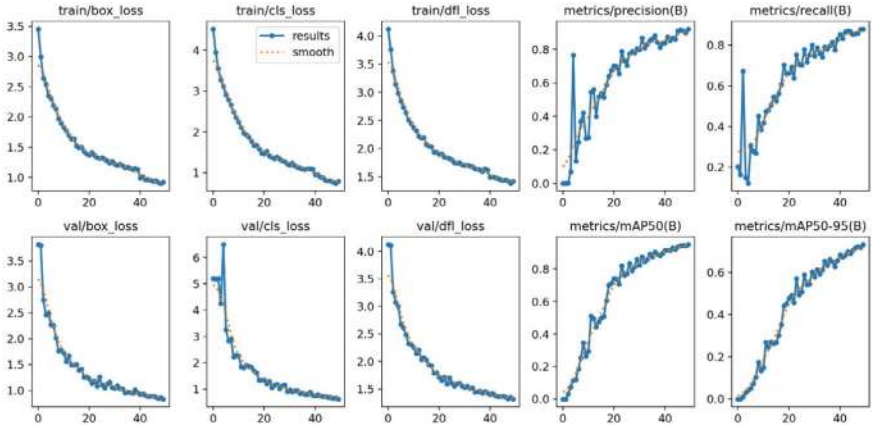
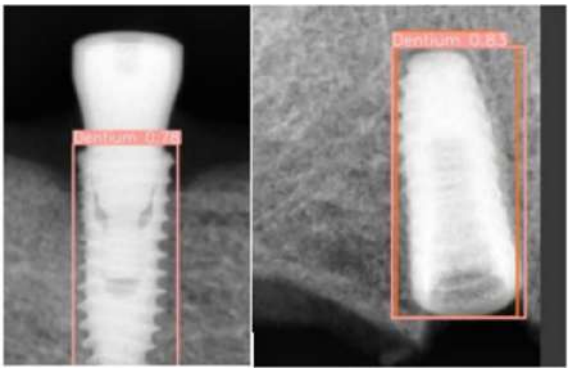


Fig. 5 Training graphs for YOLOv8

axis shows the loss value. Loss value is a measure of model performance, lower values indicate a better performance whereas higher loss values indicate poor performance. Box_loss refers to bounding box predictions, cls_loss refers to class prediction, and dfl_loss refers to the prediction of object deformations and variations. The training graphs show a consistent reduction in error with an increasing number of epochs and the model stabilized after 50 epochs; during this phase, precision was over 90%. Validation graphs show consistent improvement in precision resulting in a mean average precision over 80%. Figure 6 shows samples of model testing, and Dentium superline was identified with 78% and 83% accuracy.

Fig. 6 Testing samples



5 Discussion

Dental implants have become a well-accepted treatment alternative for missing teeth. Patients and clinicians choose implants over fixed dental prostheses due to their advantages. Implant identification is a pressing concern that needs immediate attention. With the motivation to solve this problem, we identified the potential of AI to classify an implant in a radiograph.

Image classification is the basic task of the field of computer vision which involves associating a label to a given image. Medical imaging is mainly a multi-label classification. In multi-label classification, the challenge is to ensure a bias-free and balanced dataset of all the classes [13]. In computer vision, the benchmark AI model is CNN architecture, and hence, YOLOv8 the state-of-the-art architecture was considered for the study. Among the existing studies, YOLOv8 was not trained for dental implant identification, and hence, this specific model was chosen to analyze its efficacy in implant recognition. Training an image classifier typically involves the following steps, uploading the dataset, annotation of the data, training the classifier, and evaluation of the trained model.

The model identified Adin Touareg with 94%, Osstem TSIII SA with 90%, and Dentium superline with 85% accuracy. YOLOv8 demonstrated the highest accuracy with Adin Touareg which can be attributed to the distinct and prominent features of the Adin implant system which aligns with the observations of Kim et al. [14] that the AI model can accurately identify implants with sharp features. The model exhibited confusion between Osstem TSIII SA and Dentium superline which can be attributed to some of the common features shared by them. The YOLOv8 gave an impressive overall accuracy of 89.6%. These results indicate that the YOLOv8 model can accurately detect implant systems in radiographs. The model was able to identify implant systems with good accuracy even with smaller datasets proving the potential applicability and generalizability with larger datasets.

Several studies [1, 8–11] have proved the potential of DL models in medical diagnosis using medical images indicating that DL models can recognize patterns in a medical image and can identify and classify them. This is of significance in the diagnosis of medical and dental conditions. Many studies [1, 8–11] have tested different DL models such as YOLOv3, VGG-19 (visual geometry group), GoogLeNet Inception-v3, ResNet-50, Pretrained GoogLeNet Inception, SqueezeNet, EfficientNet, Res2Next, ResNet-18, MobileNet-v2, and ResNet-50 and reported accuracies range from 71–98%. This indicates the potential of DL models, and further studies across the globe including all available implant systems would be of clinical significance.

To develop AI models, larger and well-organized datasets are crucial; however, the healthcare domain poses a unique challenge due to patients' privacy and confidentiality issues. This calls for the need for policies and support to enable researchers to access medical data to develop advanced AI models. Ethical and privacy challenges in healthcare need a striking balance between the data-driven development of advanced AI models and safeguarding patients' privacy.

Although it is imperative to use standard, clear, and sharp medical images to train AI models, the reality is that radiographs obtained from different sources, clinicians, or institutions do not meet the criteria. To overcome this bias, we feel that AI models should be trained with real-time images and larger datasets, with this it may be possible to achieve better results accounting for inherent variability in the image types encountered in clinical scenarios.

6 Limitations of the Study

These results are limited to the sample size and AI model chosen in the study. We considered only three implant systems due to the data availability concerns. Working with a wide array of implant systems and larger datasets would help in developing robust DL models. We tested only the YOLOv8 model, testing varied DL models would help to identify a robust model to be able to implement clinically.

7 Future Recommendations

More studies across the globe using patient implant images from different sources of radiographic machines and various other AI models would yield applicability and generalizability in clinical scenarios.

8 Conclusion

Implant identification in a clinical setup is crucial for the maintenance and repair of implants. DL models can be a solution to this pressing concern that holds a promise to identify implants in a radiograph. In the present study, we proved that the YOLOv8 model could accurately identify implants in radiographs even with smaller training datasets. Future work should aim toward developing robust DL models across the globe with larger and more diverse datasets to be able to apply in real-time clinical scenarios.

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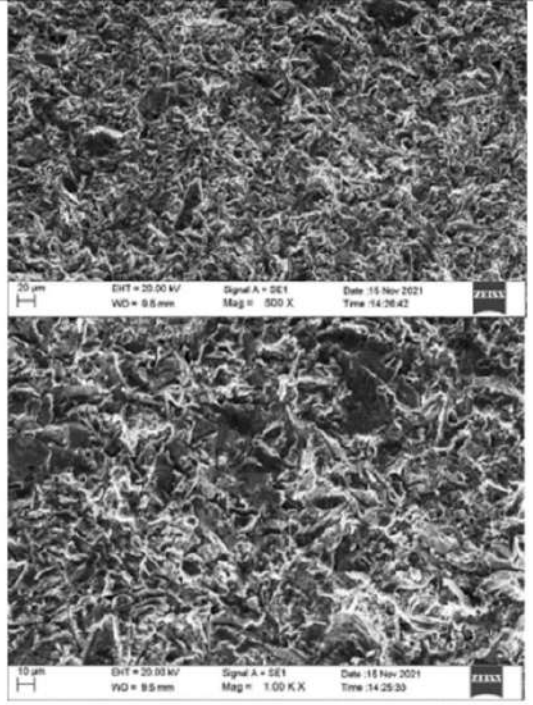


IAO-EAO-SIdP-789/PO-BR-076 | Assessment of osteogenic and anti-bacterial properties of glutathione and ascorbic acid bio-printed gel for bone tissue engineering

Khushi Gadhia*; Santosh Nelogi; Aditya Acharya; Vijay Kumbar
Prosthodontics, KLE, VK Institute of Dental Sciences, Belgaum, India

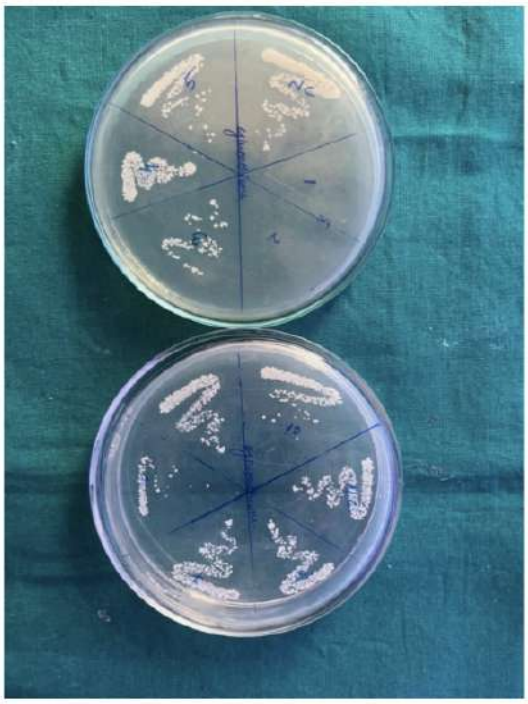
Background: Bone tissue engineering aims to develop biomaterials that can support bone regeneration while preventing infections. The use of bio-printed gels incorporating bioactive compounds such as Glutathione and Ascorbic acid offers a novel approach to enhance both osteogenesis and anti-bacterial activity.

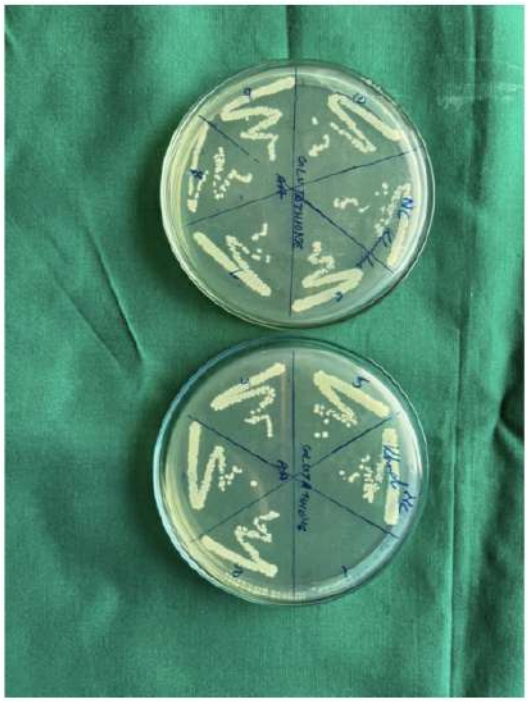
Aim/Hypothesis: This study investigates the osteogenic and anti-bacterial properties of a bio-printed gel enriched with Glutathione and Ascorbic acid for potential application in bone tissue engineering.

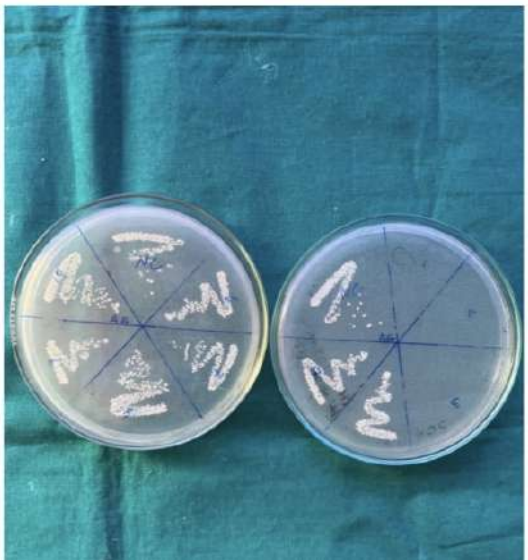
Material and Methods: A bio-printed hydrogel matrix was developed incorporating Glutathione and Ascorbic acid. MG 63 cells were seeded on the gels to assess osteogenic potential through alkaline phosphatase activity, calcium deposition assays, Anti-bacterial efficacy was evaluated using disc diffusion and minimum inhibitory concentration (MIC) assays against Aggregatibacter actinomycetemcomitans and P. gingivalis.

Results: The bio-printed gel with Glutathione and Ascorbic acid significantly promoted osteogenic differentiation of MG63 cells, as demonstrated by increased alkaline phosphatase activity, and enhanced calcium deposition, compared to control gels. Furthermore, the gel exhibited potent anti-bacterial activity, with substantial inhibition zones in disc diffusion assays and low MIC values for both Aggregatibacter actinomycetemcomitans and P. gingivalis.

Conclusion and Clinical implications: The incorporation of Glutathione and Ascorbic acid into bio-printed gels enhances osteogenic differentiation and provides effective anti-bacterial properties. These bio-printed gels hold promise for use in bone tissue engineering, offering a dual-functional approach to support bone regeneration and prevent infection







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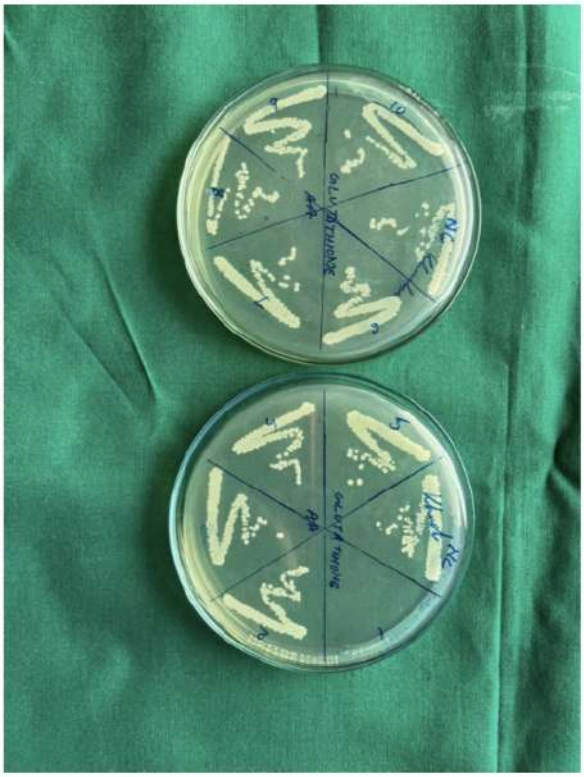

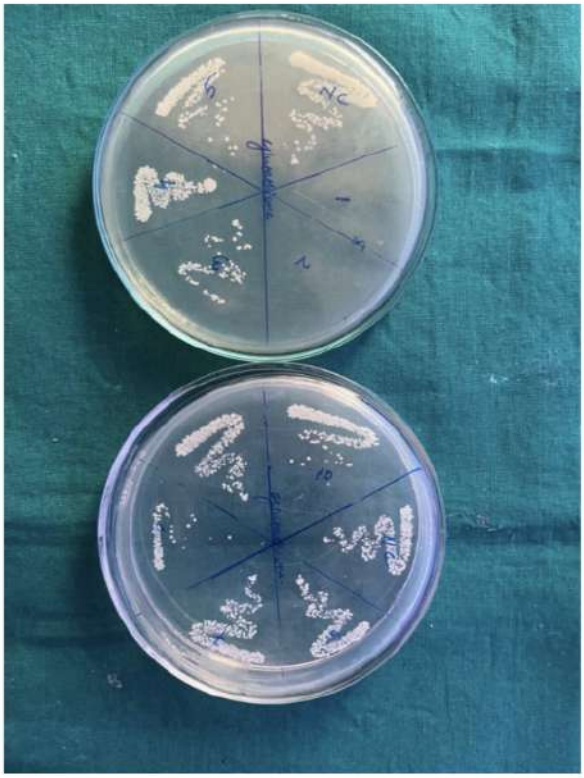

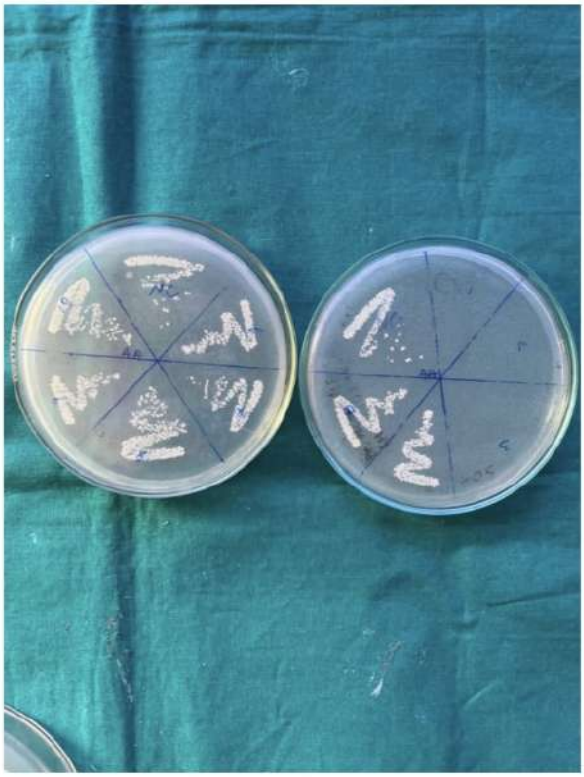

I confirm that ethical permits and approvals are in place in accordance with regulations: Yes, I confirm that ethical permits and approvals are in place.

Disclosure of Interest: None Declared.

Keywords: Bioprinting, Osseointegration, Peri-implantitis

IAO-EAO-SIdP-797/PO-BR-077 | **Impact of implant dimensions and bone quality on load-bearing ability of**

5.0...51.1 GPa elasticity-dependent cortical bone strength was used to determine 53.0...239.0 N UFL magnitudes taking into account linear correlation between the bone elasticity and strength, when experimental 100 MPa ultimate strength corresponded to 13.7 GPa of the bone elasticity modulus. For implant applicability analysis, they were compared with 120.9 N mean experimental functional load in molar site. When placed in type IV bone, 4.5×5.0 and 4.5×8.0 mm implants with cantilever reconstructions caused bone overstress resulting in implant loss.

Pathophysiological Aspects of Proteases in Cancer

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Sajal Chakraborti, Srijit Das,
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PATHOPHYSIOLOGICAL ASPECTS OF PROTEASES IN CANCER

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PATHOPHYSIOLOGICAL ASPECTS OF PROTEASES IN CANCER

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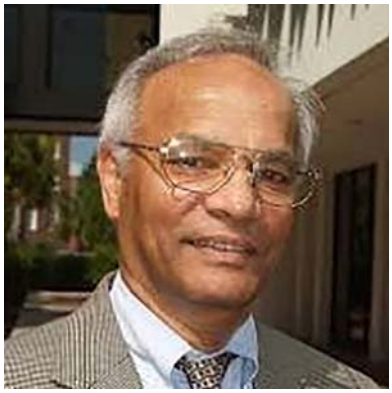

This book titled *Pathophysiological Aspects of Proteases in Cancer* is dedicated to Dr. Narendra Lal Banik, an internationally renowned neurochemist, who is a pioneer in the study and understanding of calpains in human diseases. His career has been marked by commitment to teaching and research.

Narendra Lal Banik, popularly known as Naren Banik to most of his students and colleagues, was born in the state of Bengal, India. He earned his bachelor's degree with a chemistry major from Calcutta University, Kolkata, India, in 1959. Upon completion of his bachelor's degree, he moved to London where he received his Master's degree in Biochemistry from the University of London, and subsequently joined the lab of renowned neurochemist Prof. Henry Mcllwain. Dr. Banik then pursued basic myelin chemistry studies at the Institute of Neurology, London, under the famed myelin biochemist Dr. Alan Davidson, and later at Stanford University, California, USA. In 1976, he joined the Department of Neurology at the Medical University of South Carolina, Charleston, South Carolina, and he a became full professor in 1987.

In London, Dr. Banik studied myelin and its disorders, notably the catabolic breakdown of myelin, and was devoted to the study of the catabolic breakdown of myelin by proteases. One of the early important publications in the *Biochemical Journal* in 1976 described enzymatic action employing phospholipase A and trypsin upon myelin. While working on his research projects in the mid-seventies at the Medical University of South Carolina, he observed a marked increase in extracellular calcium in the spinal cord following injury, which subsequently led him to postulate a key role of calcium-activated neutral protease, calpain, in the initiation and progression of the damage of spinal cord tissues. Subsequently, his group determined that blockade of m-calpain in the CNS was effective in the preservation of myelin after spinal cord injury.

Calpains are involved in different processes crucial for tumor progression, including cell proliferation, apoptotic cell death, survival mechanism, migration, and invasion. Calpains have aberrant expression in several human cancers; a variety of anti-cancer drugs induce cytotoxicity through the activation of calpains. The research group led by Dr. Banik observed that m-calpain expression and activity increases following glial activation, which triggers the Rho-Rock pathway. This induces inflammatory T-cell

activation and migration and mediates toxic α-synuclein aggregates as well, resulting in neuron death. Dr. Banik's research also revealed that calpains in conjunction with caspases, notably caspase-3, can cause apoptosis of the CNS cells following trauma. He found that plant-derived flavonoids can cause cell death upon inhibiting m-calpain and caspase-3 in SHSY5Y neuroblastoma cells, which causes the most common extra-cranial solid tumor in children.

Dr. Banik has published more than 450 original research publications in internationally standing journals with over 16,500 citations and D-index of 75. He is the recipient of several honors and awards. He received a Research Career Service Award from the Veterans Administration and is recognized as a Distinguished University Professor by the Medical University of South Carolina.

Dr. Banik is undoubtedly a legendary figure in the research on protease, especially calpains and caspases. A main research focus is in the field of calpain-mediated neuronal cancer pathologies. He has an excellent ability to motivate young researchers. We feel honored to dedicate this book to Dr. Narendra Lal Banik and wish him good health and success in his long, fruitful activities.

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Preface

Long recognized as destroying enzymes, proteases have drawn the interest of biochemists. The first scientific documented research entitled "The cleavage products of proteoses" was published by P.A. Levine in the first issue of the *Journal of Biological Chemistry* (October 1, 1905). Over 500,000 entries in the MEDLINE database and approximately 120 years later, proteases continue to be a major area of contemporary research interest.

Proteases have been linked to tumor growth and progression at both primary and metastatic sites, according to a number of investigations. It has been conclusively shown that the secretion of different proteases is positively correlated with the aggressiveness of the tumor. While altered tumor cells are the source of much damage, proteases of normal cells play a critical role in many biological processes. Prognosis is further complicated by some types of proteases secreted by tumor cells. Proteases are not necessarily expressed by cancer cells, and proteolytic enzymes are often expressed by tumor cells in adjacent nonneoplastic cells, and their activity is subsequently used to promote tumor growth. The identification of an organism's whole protease repertoire, or degradome, has been made easier by the recent availability of the genome sequences of various organisms. There are at least 569 proteases and homologs in the human degradome, which are divided into 5 catalytic classes: 21 aspartic, 176 serine, 150 cysteine, 28 threonine, and 194 metallo proteases. However, not all of these proteases have been connected to cancer. After a number of generations and changes, for example, in protease(s), the normal cell transforms, resulting in a localized tumor that eventually spreads to other tissues and becomes malignant.

We have presented 35 state-of-the-art articles in this book, which are divided into several sections such as (i) General aspects—where some chapters focus on the general aspects of various types of proteases in several types of cancer; (ii) Proteases as diagnostic and prognostic biomarkers—where a discernible number of chapters exposed us with the role of proteases as diagnostic biomarkers and prognostic markers in various cancer types; (iii) Protease, chemoresistance and cancer—where some chapters highlight the role of proteases in the development of chemoresistance in certain types of cancer; and (iv) Other aspects of proteases—where therapeutic aspects of some synthetic compounds have been presented.

Several contributors in this book illuminate our understanding on the role of different types of proteases with their findings, interests, and problems. We are confident that researchers engaged in cancer research will benefit out of the articles presented in the book. Some of these articles emphasize existing concepts, while some shed light on newer ideas. There is a glimmer of hope in our minds for these novel concepts.

We would like to sincerely thank each and every author for their superb writing

abilities, which are complemented by up-to-date material and make every article enjoyable to read. We like to express our gratitude to Dr. Linda Buschman, Senior Acquisitions Editor, and Dr. Barbara Makinster, Senior Editorial Project Manager, Elsevier, for their invaluable time, effort, and patience in helping us accomplish the book project's successful completion.

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SECTION I

General aspects

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CHAPTER

1

Pathophysiological aspects of calpains in cancer

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Abbreviations

b-FGF Basic fibroblast growth factor
Calpain-1 μ Calpain
Calpain-2 m-Calpain
CDK Cyclin dependent kinase
EGFR Epidermal growth factor receptor
ERK1/2 Extracellular signal-regulated kinase $^{1}/_{2}$
FAK Focal adhesion kinase
HIF1α Hypoxia inducible factor 1 alpha
ICC Interstitial cells of Cajal
NF-κB Nuclear factor kappa-light-chain-enhancer of activated B cells
NSCLC Non-small cell lung cancer
RhoA Ras homolog family member A

1. Introduction

Calpains are a family of neutral cysteine proteases. There are 17 human calpain isoform genes known to date. Among them, calpain-1 (μ-calpain, CAPN1) and calpain-2 (m-calpain, CAPN2) are relatively well studied (Kar et al., 2007, 2010; Chakraborti et al., 2012; Samanta et al., 2007; Shaikh et al., 2010). Structurally, they are the heterodimeric isoforms and possess large catalytic subunits (80 kDa), and also contain identical small regulatory subunits (CAPNS1, 28 kDa encoded by CAPN4) (Goll et al., 2003; Nian and Ma, 2021; Zhang et al., 2010).

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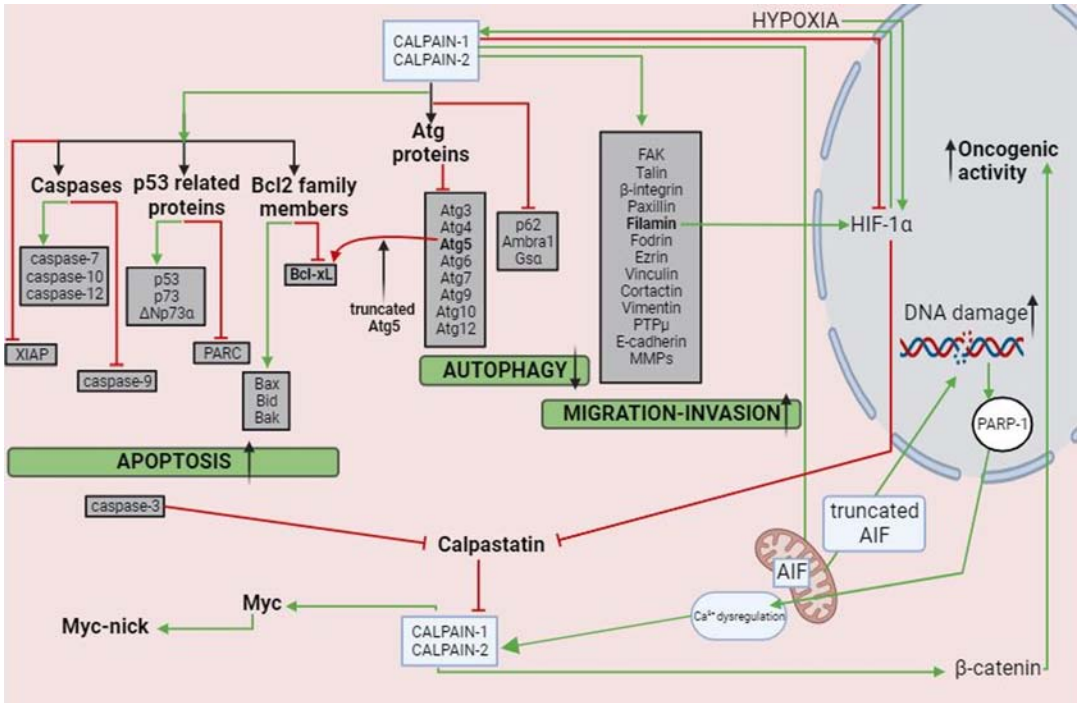

FIGURE 1.1 An outline of the substrates of conventional calpain and their involvement in the key pathophysiological processes in cancerous cells. A report is provided on a few of the mechanisms covered in the article. The macroscopic events significant for the biology of tumor cells are shown in this figure; up- or down arrows denote the general up- or downregulation of the calpain-mediated impact. Specifically, the green arrows indicate that the function/expression of the target is raised, or the transformed target takes on a fresh role, for certain processes that have been thoroughly studied mechanistically in the literature. The red inhibitor arrow denotes inhibition of the target's function or expression.

Calpains are known to be involved in many elementary physiological processes such as cytoskeletal remodeling, cell survival, and death (Shaikh et al., 2010; Kar et al., 2008, 2010). However, dysregulation of their activities may cause cancer progression via proteolysis of distinct substrates such as inhibitors of nuclear factor-κB (I-κB), focal adhesion proteins such as talin, and the protooncogenes, e.g., Myc. A considerable amount of research has indicated the involvement of the calpain–calpastatin system in cancer progression and metastasis, which interfere with cancer initiation and progression, and may develop chemotherapy resistance (Goll et al., 2003; Nian and Ma, 2021).

Fig. 1.1 is a schematic illustration of the substrates of conventional calpain and the key pathophysiological events of malignant cells involving conventional calpains. The macroscopic events significant for the biology of tumor cells are shown in this figure.

Cell migration is a multistep process involving aberrant cellular processes including chronic inflammation and tumor cell invasion and metastasis (Webb et al., 2002; Branca, 2004; Sorimachi and Ono, 2012). In these processes, the calpain system plays critical roles in the remodeling of actin cytoskeleton, cell migration, and oncogenic transformation

(Goll et al., 2003; Sorimachi and Ono, 2012). Notably, calpain-1 and calpain-2 phosphorylation by protein kinase C (PKC) and extracellular signal regulated kinase-1/2 (ERK1/2) were demonstrated to change the intrusive characteristics of cancer cells (Kimura et al., 1998). Calpain-1 mRNA and protein levels have been observed to be enhanced in renal carcinomas. An increase in calpain-2 level has also been demonstrated in colorectal cancer and non−small-cell lung cancer (NSCLC) (Nian and Ma, 2021). Calpains can act on several endogenous substrates such as cytoskeletal proteins, merlin, transcription factors, signaling components, and enzymes e.g., matrix metalloproteases (MMPs), which may promote cancer initiation and progression (Kimura et al., 1998). Representative examples for signaling molecules are protein kinase C (PKC) and focal adhesion kinase (FAK). Most notable cytoskeletal proteins are talin, desmin, paxillin, vinculin (involved in cell migration), Rac and Ras homolog family member A (RhoA), and epidermal growth factor receptor (EGFR) (involved in cell adhesion). The typical examples of the transcription factor are: p53 (tumor suppressor protein); hypoxia-inducible factor 1α (HIF1α), the protooncogenes c-fos and c-jun (modulates cell survival); p21/p27, cyclin E, and cyclin D1 (play important roles in mediating the cell cycle progression), and the exemplary members of the enzymes are caspase-3, -7, -8, -9, and -12 (necessary for cell apoptosis) (Nian and Ma, 2021; Huang et al., 2009). Among them, many act as calpain substrate proteins, which are involved in malignancies, suggesting important modulatory characteristics of calpains in cancer initiation, development, and metastasis.

Fig. 1.2 demonstrates the proteolysis of substrates involved in the advancement of cancer by calpain.

2. Colorectal cancer

Calpain-2 inhibitors have been shown to reduce inflammation induced progression of colorectal cancer (CRC) in HCT116 cells. Treatment of calpain-2 inhibitor in the cells demonstrated a marked increase in cell death, and a discernible decrease in anchorage-independent growth in the cells. Mechanistically, calpain-2 inhibition effectively reduces NF-κB nuclear translocation, which impedes tumor progression in the cells, indicating that calpain-2 inhibitor treatment could prove useful in preventing colorectal tumors that otherwise express high levels of calpain-2 (Marciel et al., 2018).

The functions of calpains are primarily concentrated on cell motility because of their involvement in regulating the cytoskeletal machinery. For example, an increase in the HCT116 CRCs occurs via epidermal growth factor (EGF)−mediated stimulation of calpain activity (Guéguinou et al., 2016). In these cells, cleavage of FAK by calpains has been observed to be vital for potent cell motility. However, calpain inhibition negatively regulates the effects of Cten, a member of the tensin (localizes to focal adhesion sites, which shares sequence homology with other tensins at its C-terminal region and that contains the SH2 and PTB domains) that promotes efficient cell motility (Konze et al., 2014). Abnormal calpain activation was shown to markedly enhance β-catenin/Wnt signaling in cells, which could profoundly increase cell survival leading to the cancer development in the cells (Konze et al., 2014). Thus, elucidation of the mechanisms by which antiapoptotic pathways are modulated by calpains for survival of the cells could provide better idea about the CRC progression and consequently novel therapeutic strategies of CRCs.

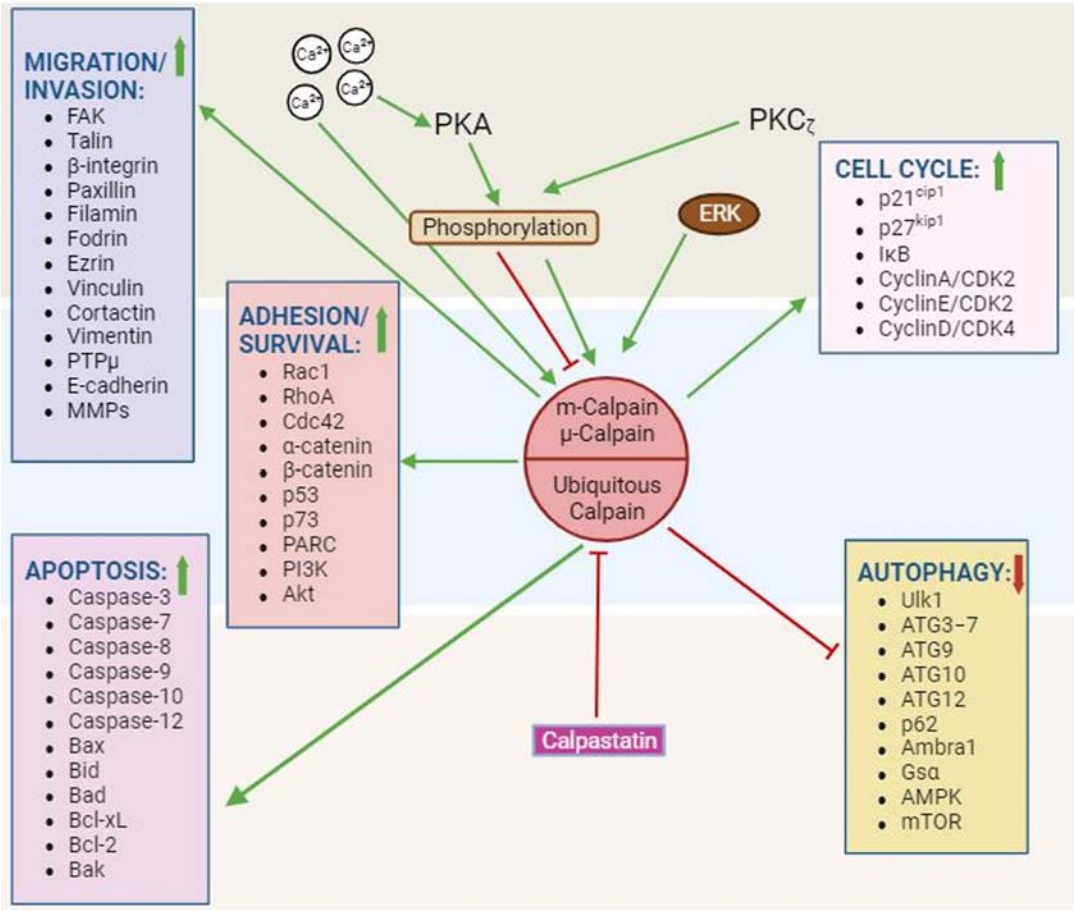

FIGURE 1.2 Calpain-induced proteolysis of substrates important in the development of cancer. m-Calpain (CAPN2), μ-calpain (CAPN1), and other calpains are activated in the presence of varying calcium ions concentration. Their endogenous inhibitor, calpastatin, can inhibit their activity in the presence of calcium and PKA-mediated phosphorylation. Numerous medications, including genistein, cisplatin, and resveratrol, as well as phosphorylation mediated by PKCζ and ERK, can cause calpain activation. During the process of carcinogenesis, calcium-dependent proteins play a crucial role in controlling several cell processes such as the cell cycle, cellular migration and invasion, adhesion and survival of cells, autophagy, and apoptosis. AMPK, AMP-activated protein kinase; ATG, autophagy-related protein; PKB, protein kinase B; Bcl-2, B cell lymphoma 2; Bcl-xL, B cell lymphoma-extra large; Bad, Bcl-2-associated death promoter; Bak, Bcl-2-antagonist/killer; Bax, Bcl-2-associated X protein; Bid, BH3-interacting-domain death agonist; Cdc42, cyclin-dependent kinase; ERK, extracellular signal-regulated kinase; FAK, focal adhesion kinase; Grb2, growth factor receptor-bound protein 2; and Gsα, G protein Sordaria alpha subunit; p21cip1, cyclin-dependent kinase inhibitor 1A; p27kipl, cyclin-dependent kinase inhibitor 1B; p53, tumor protein p53 (TP53); p62, sequestosome 1 (SQSTMI); p73, tumor protein p73; PARC, p53-associated Parkin-like cytoplasmic protein; IκB, inhibitor of NF-κB; MEK, MAPK/ERK kinase; MMP, matrix metalloproteinase; mTOR, mammalian target of rapamycin; PKA, protein kinase A; PKC_i, protein kinase C iota; PTPμ, protein tyrosine phosphatase μ; PI3K, phosphatidylinositol 3-kinase; RhoA, ras homolog gene family, member A; RTK, receptor tyrosine kinase; Shc, Src homology and collagen; SOS, son of sevenless; Ulk 1, Unc-51-like autophagy activating kinase 1; Racl, Ras-related C3 botulinum toxin substrate 1; Raf, rapidly accelerated fibrosarcoma are used in this figure.

3. Renal cell carcinoma

Renal cell carcinoma (RCC) is a universally occurring tumor of the urological system and is categorized as the second in urological cancer mortality (Potz et al., 2016). Higher Capn4 mRNA and protein levels were found in advance stage of ccRCC patients (Zhuang et al., 2014). However, expression of other isoforms of Capn did not elicit discernible invasive abilities in ccRCC cells.

Focal adhesion kinase (FAK) activation has been observed in a variety of tumor cells, which can be correlated with the malignancy and invasiveness of human RCC. Importantly, FAK phosphorylation activates NF-κB, and NF-κB subsequently mediates RCC progression. The NF-κB family consists of five members: RelA (p65), RelB, c-Rel, NF-κB1 (p105/p50), and NF-κB2 (p100/p52) (Khan et al., 2012). The NF-κB pathway has been shown to be involved in many facets of RCC progression such as angiogenesis, invasion, metastasis, and multidrug resistance (Morais et al., 2011). Capn4 activates NF-κB signaling pathway and stimulates RCC cell proliferation through activating the FAK signaling pathway. Immunostaining of p50 and p65 in the RCC cells has demonstrated a correlation between invasion and metastasis of RCC and the expression and activation of NF-κB (Oya et al., 2003).

Focal adhesions are localized where integrin clusters are formed at the cell−extracellular matrix junctions. FAK, a nonreceptor tyrosine kinase, has been observed at focal adhesions and elicits signaling as well as scaffolding functions (Zhuang et al., 2018). Capn4 was shown to cause phosphorylation of specific tyrosine residues of FAK and subsequently induce increase in NF-κBp65 phosphorylation. Furthermore, Capn4-mediated cell proliferation of RCC cells requires upregulation of NF-κBp65 phosphorylation via stimulation of FAK-Akt-MAPK signaling axis (Zhuang et al., 2018).

An increase in the expression of Capn4 has been shown to enhance the protein levels of MMP-2, vimentin, and N-cadherin, while this downregulates E-cadherin. Capn4 silencing attenuates MMP-2, vimentin, N-cadherin, but this upregulates E-cadherin protein levels in the cells. Capn4 has also been demonstrated to induce an increase in cancer metastasis, which suggests that Capn4 is associated with ccRCC invasion and migration, which eventually promotes cancer metastasis in ccRCC (Zhuang et al., 2018).

MMP-2 has been observed to be involved in the pathogenesis of several types of cancer. Importantly, MMP-2 has been demonstrated to play a key role in ccRCC metastasis. Knockdown of CAPN4 was shown to attenuate the MMP-2 protein expression in nasopharyngeal carcinoma (Zheng et al., 2014). Additionally, Capn4 overexpression causes a marked increase in MMP-2 activity in intrahepatic cholangiocarcinoma cells (Xu and Deng, 2006). Moreover, Capn4 silencing decreases MMP-2 expression and activity in ccRCC cells. Thus, Capn4 seems to trigger tumor metastasis in ccRCC through increase in MMP-2 expression (Xu and Deng, 2006).

4. Hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is the largest type of liver malignancy and is also the third cause of death worldwide. Capn4 was found to be the major gene, which increases

metastasis of HCC. Capn4 has been observed to be physically associated with FAK and stimulates the FAK-Src axis through an increase in the phosphorylation of the tyrosine residues of FAK, Src, and p130Cas/BCAR1. Capn4 knockdown has been shown to suppress the malignant characteristics of HCC cells and also attenuates the FAK-Src signaling pathway. Besides, Capn4-caused invasion and metastasis of HCC cells require a substantial increase in the MMPs activity upon stimulation of this signaling pathway (Dai et al., 2014).

Phosphorylation of FAK at Tyr-397 and other proteins such as Src and p130Cas are necessary for focal adhesion formation, cell migration, and invasion (Hsia et al., 2003). Inhibition of FAK in HCC cells with an increase in the level of Capn4 was shown to attenuate cell proliferation and invasiveness, indicating that high levels of Capn4 favor tumor initiation, development and metastasis, conceivably by increasing FAK tyrosine phosphorylation and subsequently stimulation of the FAK-Src-Cas signaling axis. In this context, it is worthy to mention that MMP-2 and MMP-9 are involved in regulating the FAK-Src signaling axis—induced initiation and progression of HCC. MMP-2- and MMP-9-mediated mechanisms in this scenario have been suggested to be different. MMP-9 seems to act as a switch for tumor angiogenesis (Ganguly et al., 2013). C-Jun binds to the AP-1 site in the MMP-9 gene promoter, thereby enhancing MMP-9 expression (Oya et al., 2003). However, unlike MMP-9, MMP-2 could be one of the many Src targets that are modulated, at least partly, by FAK-dependent mechanism (Zhang et al., 2002).

The signal initiated by Capn4 stimulates FAK-Src signaling axis, which help in the enhancement of the binding of Sp1 to MMP-2 gene, thereby inducing increase in MMP-2 expression (Kuo et al., 2006). Additionally, the SH domain of p130Cas, an important constituent of the FAK protein complex, is stimulated by Capn4 and that is an important aspect of MMP-2 activation (Brábek et al., 2005; D'Aguanno et al., 2021) have observed that hypoxia-inducible factor (HIF) triggers melanoma invasion and invadopodia generation via platelet-derived growth factor receptor (PDGFR) and FAK-dependent activation of Src, and by interrelating ECM degradation through increase in MT1-MMP and MMP-2 activities. Thus, a marked increase in the stimulation of MMP-2 through FAK and Src activation both are important in Capn4 mediated growth and invasion of HCC cells.

Fig. 1.3 is an illustrative diagram showing how the FAK signaling pathway is activated by Capn4 that leads to HCC.

Inhibition of FAK-Src signaling axis attenuates MMP-2 expression and subsequently inhibits proliferative and invasive capacity of HCC cells (Dai et al., 2014).

Liver transplantation (LT) is one of the best therapeutic options for nonresectable hepatocellular carcinoma (HCC). The CAPN4 overexpression underlies invasion and metastasis after LT for HCC and that seems to be a biomarker that could be used for diagnosis and a target for therapy (Dai et al., 2014).

5. Gastric cancer

The underlying mechanisms associated with the initiation and the progression of gastric cancer (GC) are of significance for its diagnosis and eventually for treatment. Calpastatin, a component of the calpain system, has been suggested to be a critical factor in

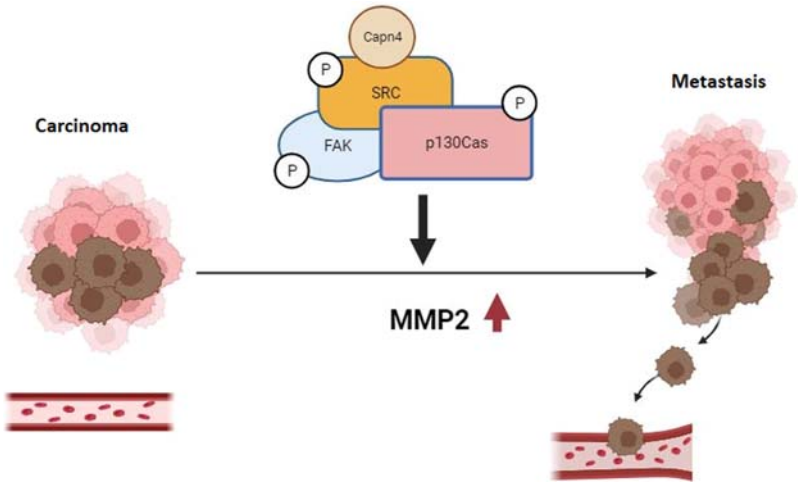

FIGURE 1.3 A prospective schematic illustration depicting how Capn4 contributes to HCC by triggering the FAK signaling pathway. Capn4's physiological role involves physically interacting with FAK to increase the signaling pathway's activation. Thus, the overexpression of Capn4 in HCC may be involved in the development and spread of tumors, potentially through the activation of the FAK signaling pathway through the start of the FAK-Src-Cas complex tyrosine phosphorylation cascade (together with increased expression and activity of MMP2).

the tumorigenesis and migration of GC (Liu et al., 2017). Capn2 protein level has been observed to be enhanced in GCs in comparison with normal tissues, while calpastatin protein level decreases; as a consequence, no change in Capn4 level has been observed (Liu et al., 2017).

GC poses a considerable public health concern since it is currently regarded as the fourth most common type of cancer and the third most common cause of death worldwide. To better understand the role of calpain and calpastatin in GC, the levels of Capn1, Capn2, and calpastatin expression were compared in human GC tissues and normal glandular tissues. Capn2 protein level has been observed to be enhanced in GC in comparison with the normal tissues. In contrast, calpastatin protein level decreases and calpain-1 level remains unchanged indicating that expression of these proteins does not play a role in the clinical variables of GC (Liu et al., 2017).

5.1 Inflammatory bowel disease

One of the major roles of calpain-2 in inducing the activation of macrophages and inflammation could be due to secretion of cytokines, and the critical part of the increase in the level of calpastatin is to attenuate calpain-triggered inflammatory bowel disease (Huang et al., 2013). Calpain-2 inhibitors have been shown to attenuate both colitis and also the colorectal cancer. The effect of the inhibitor was found to be due to the inhibitory kB (IκB) to reduce nuclear translocation of NF-κB (Guéguinou et al., 2016), which was shown to be inhibited by calpain-induced cleavage of IκB. Results deduced from in vitro and in vivo observations

inferred a correlation between calpain-2 level and feebleness of calpain-2 inhibitor therapy for suppression of colon cancer (Marciel et al., 2018).

Calpain-2 has been suggested to be critical for drug resistance in colorectal cancer. Notably, CPT-11 (irinotecan, a drug used alone or in combination with other drugs to treat metastasis of CRC) is known to be the first-line chemotherapy for advanced stage of colorectal cancer, albeit a considerable number of patients grow resistance upon prolonged treatment (Siegel et al., 2014). The contributing factors associated with the resistance are complex. It has been suggested that the HT-29 human colon cancer cells exposed to CPT-II led to drug-resistant tumors with upregulation of calpain-2 protein expression as well as activity (Fenouille et al., 2012). Calpain-2 has been observed to directly cleave IκB, leading to increase in NF-κB activity in the HT-29R cells. It has been proposed that calpain-2-mediated IκB degradation provides CPT 11 secondary resistance in CRC. High levels of calpain-2-mediated protumor effects were shown to occur upon association of the enzyme with Ca^{2+} for activation. During the transformation of healthy cells of CRC, Ca^{2+} homeostasis is modulated in a process known as Ca^{2+}-induced remodeling (Villalobos et al., 2017). In fact, numerous types of cancer showed a marked change in intracellular Ca^{2+} homeostasis, which has been observed in angiogenesis, tumor initiation, development, and metastasis (Xie et al., 2016). This prompted scientists to evolve Ca^{2+} chelators, inhibitors, or regulators as anticancer drugs. However, heterogeneity of Ca^{2+} regulatory mechanisms in several types of cancer evidently restricts a broad application of chemotherapeutics that target store-operated Ca^{2+} entry in different types of cancer cells (Hooper et al., 2016). Calpain-2 has been shown to be efficacious target for some types of colorectal cancer (Rose et al., 2015). Screening tumor biopsy samples or surgical resections for determining the levels of calpain-2 appear to be a novel approach for providing precision medicines for colon cancer treatment. Therefore, calpain-2 levels could be a useful biomarker to envisage the role of calpain-2 inhibitors in cancer therapy (Marciel et al., 2018).

6. Breast cancer

Breast tumors express low-molecular-weight isoforms of cyclin E, which are involved in increased kinase activity and higher metastatic potential with aggressive behavior. An increase in cyclin E expression in MCF7 cells increases calpain-2 level, thereby promoting proteolysis of cyclin E along with numerous calpain substrates such as FAK, calpastatin, pp60erc, and p53. Calpain-2 inhibition attenuates cyclin E proteolysis, whereas its stimulation induces increase in cyclin E proteolysis in the MCF7 cells. In human breast tumors, high levels of cyclin E were found to be synchronous with the increase in calpain-2 protein and proteolysis of FAK as well (Libertini et al., 2005).

An increase in calpain-2 activity could trigger proteolysis of FAK, a calpain substrate, to produce a 90-kDa form, which can be cleaved to a 90-kDa form (Cooray et al., 1996). FAK has been shown to play a pivotal role in coordinating cell adhesion and migration. It has been demonstrated that upon proteolysis of FAK in platelets, its activity was attenuated, resulting in its detachment from the cytoskeleton. Notably, cells that are devoid of FAK exhibit a marked decrease in the mobility of the cells, which could also play a critical part

in the recruitment of Capns into focal adhesions (Ilić et al., 1995); thereby, it can mediate proteolytic cleavage of other focal adhesion proteins that subsequently are able to modify cell adhesion and migration (Carragher et al., 2003).

Cyclin E is the major component that regulates the cyclin/cyclin dependant kinase (CDK) complex; cyclin E has been shown to play vital roles in several types of tumors (Keyomarsi et al., 2002). The involvement of cyclin E in tumorigenesis of breast cancers has been well documented (Span et al., 2003). The overexpression of cyclin E has been shown to be associated with proteolytically processed more active low-molecular-weight (LMW) isoforms (Span et al., 2003; Porter et al., 2001), which are not inhibited by the CDK inhibitors p21 and p27 and, therefore, possess higher CDK kinase activity (Akli et al., 2004). Thus, cyclin E-mediated dysregulation of calpain-2 appears to be an important phenomenon in the initiation and development of breast cancer (Cooray et al., 1996). Cyclin E expression and calpain-2 concentrations have also been correlated in human breast cancer, which elicit aggressive behavior and poor clinical prognosis (Cooray et al., 1996).

Calpain also cleaves proteins, which play critical roles in transcriptional regulation, for example, p53. Calpain-2 has been shown to cleave p53 at the NH2 terminal region resulting in the formation of the 48-kDa p53 isoform in ubiquitin-independent manner, leading to inhibition of tumor suppression (Kubbutat and Vousden, 1997). Upon overexpression of cyclin E, a meager amount of the native p53 was found to be expressed, albeit the 48-kDa proteolytic form was discernibly detected. Notably, the total amount of p53 was reduced, which suggests that calpain significantly mediates the proteolysis of p53. The decrease in the p53 level could enhance transversal of DNA damage checkpoints, thereby providing genomic instability, which modifies apoptotic responses, and trigger tumorigenesis (Libertini et al., 2005).

In breast cancer, inhibition of O-linked N-acetylglucosamine (O-GlcNAc)-mediated protein modification occurs in muscle via extracellular vesicle−encapsulated miR122 that targets O-GlcNAc transferase (OGT). Notably, O-GlcNAculation of the ryanodine receptor 1 (RyR1) competes with NEK-10-dependent phosphorylation and enhances K48-associated ubiquitination and subsequently enhances proteasomal degradation (Yan et al., 2022) . NEK10 (INIMA-related kinase 10) has been shown to phosphorylate tyrosine residues of p53 and thereby regulate its transcriptional activity. Upon binding with Raf-1 and MEK1, Nek10-mediated MEK1 activation has been observed to be increased. The miR-122-dependent decrease in OGT enhances RyR1 in breast cancer cells. The muscular protein O-GlcNAcylation has been observed to be regulated by hypoxia-induced increase in HIF-1A-dependent OGT promoter activation. In this kind of malignancy, inhibition of O-GlcNcylation that occurs via increasing RyR1 could lead to higher $[Ca^{2+}]_i$ level, which activates calpains and subsequently cleaves desmin filaments, leading to the loss of skeletal muscle mass and myofibrillar destruction (Yan et al., 2022).

In BC patients, muscle proteolysis and atrophy seem to be important in cachexia, which support tumor growth via release of a huge amount of amino acids release into the circulation (Argilés et al., 2014). The existence of low muscle mass and poor muscle quality has been observed in over one-third in nonmetastatic BC patients (Caan et al., 2018) and about two-thirds in metastatic BC patients (Rier et al., 2017), indicating that BC patients with reduced muscle mass have a greater risk of death (Alderton and Steinhardt, 2000).

7. B cell chronic lymphocytic leukemia

B cell chronic lymphocytic leukemia (B-CLL) is the predominant type hematological cancer in the group of middle aged/elderly population among the Caucasians (Amaya-Chanaga and Rassenti, 2016). B-CLL cells exhibit elevated activity of calpain-1, which is known to control proliferation and apoptosis of the cells. A significant correlation exists between the amounts of intracellular calpain-1 and clinical staging of the disease, with RAI stage 1 (a high number of lymphocytes and enlarged lymph nodes) in the leukemic cell. Besides, a significant correlation also exists between calpain-1 and phosphorylated (p)ZAP-70 in B-CLL lymphocytes (Łopatniuk et al., 2020). An increase in the ZAP-70 expression can cause a chronic lymphocytic leukemia subtype, where immunoglobulin genes were not mutated. Calpain activity in the B-CLL cells was found to be associated with diminished activities of proapoptotic caspase-3 and -9, and reciprocally correlated with an increase in the level of the antiapoptotic protein, Bcl-2. Thus, calpain-1 activity in B-CLL cells seems to be a prospective target that could modify the characteristic properties of these cells and facilitates therapy (Witkowski et al., 2002).

8. Oral squamous cell carcinoma

Calpain-1 has been observed to be overexpressed in oral squamous cell carcinoma (OSCC) cells. OSCC is a highly variable disease with heterogenous genetic and epigenetic changes, which are responsible for ~50% of the malignant oral abrasion (Storr et al., 2011; Storr et al., 2011).

Calpain-1 overexpression was demonstrated in OSCC cells and tissues (Storr et al., 2011; Storr et al., 2011; Storr et al., 2012). Deletion of calpain-1 could be related to an inhibition of the migratory and invasive capacity of the OSCC cells. Calpain-1 has been observed to regulate the expression and the resultant activation of MMPs (Popp et al., 2003). Importantly, MMPs can proteolytically degrade several extracellular matrix components, thereby paving the way for invasiveness of the OSCC cells. Thus, presenting calpain-1 with its discrete inhibitors could probably attenuate the spreading of OSCC cells. However, the cells exhibit no change in the ameboid migration and invasion upon treatments with the inhibitors. Calpain inhibitors also promote HT1080 cell (epithelial cells derived from connective tissue from a patient with fibrosarcoma) invasion, which is inhibited by a Rho-related protein kinase inhibitor, thereby decreasing ameboid formation and resultant invasion (Carragher et al., 2006; Ma et al., 2017). At present, no clear explanation in this scenario has, however, been available, and further research is needed in different model systems (Ma et al., 2017). A detailed and comprehensive analysis of the physiological roles of calpain family members in the context of complex cellular or in vivo systems is required, and that warrants further investigation.

9. Pancreatic cancer

A single-nucleotide polymorphism of calpain-10 (CAPN10) was found to be related to the development of pancreatic cancer among smokers (Fong et al., 2010). In the normal

pancreatic tissue, calpain elicits a number of functions such as Ca^{2+}-induced insulin secretion and β-cell dissemination (Parnaud et al., 2005). Besides, a change in the calpastatin and calpain balance has been observed to play a role in acute pancreatitis in rats (Weber et al., 2004). The calpain system also plays an important role in tumor progression through different pathways that have been implicated in cellular migration, apoptosis, and cell survival (Storr et al., 2011; Storr et al., 2011). Scanty information is available regarding function of the calpain system in pancreatic, bile duct, and ampullary cancers. A single-nucleotide polymorphism in calpain-10 (CAPN10) was observed to be responsible for the development of pancreatic cancer in smokers (Fong et al., 2010). Additionally, low calpain-2 level has been shown to be related to the poor longevity of the patients with pancreatic cancer (Storr et al., 2011; Storr et al., 2011). Currently, it remains to be determined whether low levels of calpain-2 protein expression are related to the survivability of pancreatic cancer patients, albeit numerous factors such as the tumor type, the chemotherapy treatment, or related organ(s) and relevant cell specificity could play a critical role in this scenario.

10. Calpain-6 and cancer and stem cells

Biochemical mechanism associated with sarcoma formation is currently not clearly known. Cancer stem cells are known to play vital roles in sarcoma development, but lack of understanding about the critical markers poses difficulties to gain better insights about their functional characteristics. Calpain-6 expression has been observed to be associated with the features of cancer stem cell. It has been demonstrated that unique tumor initiation and metastatic abilities exists in calpain-6 expressed mouse models of bone sarcoma. Calpain-6 levels are relatively high in tumors, which could help in the propagation of mouse to establish patients acquired xenografts. Calpain-6 levels have been shown to be increased by hypoxia, a condition that is known to be prevalent in tumors. The hypoxic environment facilitates chemoresistance and hostility of tumor cells (Höckel and Vaupel, 2001). Accordingly, calpain-6 expression was found to be enhanced when O_2 levels are low. Additionally, calpain-6 appears to be necessary for hypoxia-mediated attenuation of senescence. Thus, an increase in calpain-6 level could be required for specific stem cell's adaptation to the tumor environment (Höckel and Vaupel, 2001). Besides, calpain-6 level depends on the stem cell transcription network involving Oct4, Nanog, and Sox2, which are known to be activated in hypoxic environment. Calpain-6 knockdown prevents tumor progression in mouse and attenuates the number of cancer stem cell inhabitants. The malignancy development has been observed to be associated with the appearance of cell clones with cancer stem cell (CSC) characteristics related to the genetic modifications, epigenetic regulation, and specialized environments (Eide and Druker, 2017). The presence of calpain-6 was demonstrated in osteosarcomas, and its level is elevated during reappearance of bone tumors. Additionally, calpain-6 expression in cancer cells is related to the inhibition of apoptosis and chemoresistance (Marion et al., 2012). Thus, calpain-6 expression seems to be important for progression of primary malignant tumors (Glinsky, 2008).

Data from transcriptomic analyses revealed that calpain-6 level is negatively correlated with senescence markers. Calpain-6 knockdown has been observed to suppress hypoxia-mediated inhibition of senescence entry and also promotes autophagy. Thus, calpain-6 could play critical roles in this sarcoma cells with stem-like characteristics upon activation by hypoxia-inducing factor—α (HIF-α) and, thereby, inhibits autophagy and sustains the tumor-initiating cell population. These observations suggest a novel therapeutic approach for targeting sarcoma stem cells (Andrique et al., 2018).

11. Cervical cancer and calpain

Among the calpain family members known to date, calpain-6 has the characteristics that it does not possess the calmodulin-like domain, which are observed in the C-terminus of other calpains (Kar et al., 2010). This supports the fact that calpain-6 does not elicit proteolytic activity or that it utilizes a separate location for proteolysis, depending on the systems. Calpain-6 has been observed to be elevated in uterine cervical cancer tissue in comparison with normal tissue. In ovarian and cervical cancer cells and in tissues, calpain-6 induces a marked increase in cervical cancer, which suggests that calpain-6 promotes tumorigenesis upon inhibiting apoptosis and expedite angiogenesis (Rho et al., 2008).

Two events have been suggested to be primarily important for initiation and development of cancer cells. One is resistant to apoptosis, and the other promotes angiogenesis. To determine the role(s) of calpain-6 in tumorigenesis, calpain-6 has been overexpressed and inhibited in human cervical cancer cells (HeLa cells) and then determined the cell viability and apoptosis in the presence of cisplatin, which is widely used to treat various types of cancer. Cisplatin dose-dependently inhibits proliferation of the HeLa cells (Wsierska-Gdek et al., 2002). Some human cervical cancer cell lines such as SKOV-3 and Caski have been observed to be unperturbed upon cisplatin treatment (Rho et al., 2008). The abnormal calpain-6 level has been observed to save HeLa cells from cisplatin-triggered apoptosis. Since cisplatin-mediated apoptosis occurs via the caspase-3-mediated pathway (Blanc et al., 2000); its activity was measured in cisplatin-treated HeLa cells, with and without the inhibitors of calpain-6. The increase in calpain-6 causes stimulation of caspase-3 in HeLa cells. Thus, calpain-6 is involved in tumor cell apoptosis, the mechanism of which is different from that observed for calpain-1 and -2 (Ray et al., 2000).

The atypical calpain-6 structure could provide some clues to understand mechanistic aspects by which calpain-6 attenuates caspase-3 activity in cisplatin-treated HeLa cells. Calpain-6 is devoid of calmodulin-like domain present in the C-terminus of other calpains (Dear et al., 1997), suggesting that calpain-6 may not have proteolytic activity or that it utilizes a different active site. One probable explanation could be that calpain-6 emulates with calpain-1 and -2 for the same substrates, thereby competitively inhibiting their proapoptotic activity. This process may limit the availability of the substrates that have already been used to a large extent by calpain-1 and -2, thereby attenuating calpain-6 activity during the apoptotic phenomena. An alternate mechanism could be that calpain-6 inhibits the other family members by associating with them. In this situation, the ligands that bind calpain-6 could not adequately participate to catalyze proapoptotic cleavage activities, since calpain-6 is

devoid of proteolytic activity. In agreement to this possibility, calpain-1 and-2 are able to make heterodimers with calpain 4, which contains a 30-kDa regulatory subunit and an 80-kDa catalytic subunit. During the activation process, the 30-kDa subunit finally yields a 17-kDa form, while the 80-kDa subunit turned to a 76-kDa enzymatically active form (Kitagaki et al., 2000). Further study will clarify whether calpain-6 competitively inhibits calpain-1 and -2 during apoptotic processes or whether calpain-6 forms heterodimers with other calpains.

Angiogenesis is primarily the process for producing new blood vessels from preexisting vessels. It is an important aspect in tumor growth and progression, since this process allows the growing tumor to receive oxygen and nutrients (Yancopoulos et al., 2000). An important mediator that enables blood vessel formation is vascular endothelial growth factor (VEGF). VEGF promotes endothelial cell proliferation and subsequently its migration. Endothelial cells play numerous roles such as tumor growth and maintenance in different organs and tissues. Since calpain-6 takes part in microtubule stabilization and cytoskeletal organization (Tonami et al., 2007), it seems important to explore the contribution of calpain-6-mediated angiogenesis in human umbilical vein endothelial cell (HUVEC) migration and invasion and tube formation. The HUVECs produce capillary-like structure upon activation of calpain-6, which occurs in the cells upon receiving angiogenic signals. The observation that an increase in calpain-6 expression in vesicular endothelial cells during tumor growth and progression has not been adequately appreciated. However, VEGF and b-FGF have been shown to induce intracellular Ca^{2+} signals in endothelial cells, which promote angiogenesis (Patton et al., 2003). This growth factor—promoted intracellular Ca^{2+} signaling may also trigger calpain-6 activity in the cells.

12. Nasopharyngeal carcinoma

A large number of researches have demonstrated that deficiency of Capn4 may lead to dysfunction of calpain-1 and calpain-2 in a variety of cells and tissues (Arthur et al., 2000). Capn4 knockdown has been shown to inhibit migration and also decreases focal adhesion of fibroblast cells (Zheng et al., 2014). Capn4 mRNA and protein levels were found to be elevated in human nasopharyngeal carcinoma (NPC) tissues in comparison with normal NP tissues (Zheng et al., 2014). Besides, Capn4 was found to be correlated well with advanced stages of Epstein—Barr virus infection. Capn4 siRNA knockdown attenuates cell migration and invasion in NPC cells and tissues. This seems to be due to the Capn4 downregulation associated with a marked decrease in the expression of matrix metalloproteases-2 (MMP-2), snail, and vimentin. Moreover, Capn4-mediated upregulation of MMP-2 occurs via activation of NF-κB due to an increase in the phosphorylation of p65, a subunit of NF-κB. Thus, Capn4 may be considered as a prognostic factor and a prospective therapeutic target in NPC (Zheng et al., 2014).

Among several genes that are involved in tumor metastasis, MMP-2, MMP-9, snail, vimentin, E-cadherin, H-cadherin, and β-catenin are substrates for proteolysis by calpains, where Capn4 plays the predominant role in maintaining the stability and activity of the cysteine protease (Undyala et al., 2008). MMP-2 plays a pivotal role in the pathogenesis of several types of cancer, for example, metastasis of NPC (Zheng et al., 2014). MMP-2 mRNA and

enzyme levels are prominently inhibited upon Capn4 knockdown by its siRNA in 5-8F cells (5-8F: a subline of SUNE-1 nasopharyngeal carcinoma cells, which have discernible metastatic and tumorigenic abilities) and CNE2 cells (CNE2: epithelial cell line isolated from imperfectly differentiated nasopharyngeal carcinoma). MMP-2 siRNA has been shown to inhibit invasion of 5-8F and CNE2 cells. In agreement with the aforementioned, it has been observed that Capn4 enhances MMP-2 expression in ICC cells (ICC: interstitial cells of Cajal., which are found in the circular muscle. These are spindle-shaped cells associated with the long axis of the surrounding smooth muscle cells (Zhang et al., 2013), indicating that Capn4 could increase invasion and metastasis of NPC cells by increasing MMP-2. Additionally, overexpression of MMP-2 in Capn4 siRNA-transfected 5-8F and Capn4 siRNA of CNE2 cells reverted their invasiveness. Thus, MMP-2 appears to be a downstream target in Capn4-induced increase in the metastasis of NPC cells (Zheng et al., 2014).

The NF-κB signaling axis has been observed to play a critical role in several pathophysiological processes such as inflammation, apoptosis, cell migration, and cell cycle control, which are frequently dysregulated in many types of cancer (Yang et al., 2013). Notably, NF-κB can promote the generation of MMPs in tumor cells and the surrounding mesenchymal cells as well, which contributes to the degradation of extracellular matrix during tumorigenesis. In NPC cells, NF-κB promotes the invasion process to stimulate tumor development (Sun et al., 2012). Downregulation of Capn4 by siRNA markedly decreases phosphorylation of p65 component of the NF-κB p65 subunit, while Capn4 overexpression triggers p65 phosphorylation in NPC cells. Moreover, the NF-κB inhibitor, helenalin attenuates MMP-2 expression that occurs during Capn4 overexpression. Thus, Capn4 can modulate metastasis of NPC cells via NF-κB-mediated increase in the level of MMP-2. Capn4 also promotes activation of the NF-κB-dependent pathway, indicating that MMP-2 and Capn4 may cooperate in the initiation and progression of NPC pathogenesis (Zheng et al., 2014).

13. Glioblastoma multiforme

Glioma is an aggressive tumor and is difficult to treat. The median survival time of patients with glioma is only about 15 months (Stylli, 2020). An increase in the Capn4 expression can be correlated with glioma tumorigenesis, whereas its decrease in glioma cells was shown to be associated with inhibition of the invasion and migration of glioma cells. Thus, Capn4 can be used as a prospective therapeutic target for glioma therapies (Cai et al., 2014).

Surgical resection followed by radio- and chemotherapy with temozolomide (TMZ) are the common treatment procedures associated with glioblastoma multiforme (Strik et al., 2012). During TMZ therapy, tumor and tumor stem-like cells often develop chemoresistance (Singh et al., 2021). Thus, it seems important to identify the pathways related to the increase in TMZ resistance. Conceivably, these pathways are associated with an increase in the abilities of DNA repair, inhibition of apoptosis, and alteration of cellular metabolism.

14. Osteosarcoma

Patients with malignant diseases are normally suffering from cancer-induced bone pain (CIBP). This affects the patients with primary bone sarcomas and bone metastasis (Colvin and Fallon, 2008). The pain occurs mainly in three states: background pain, spontaneous pain, and movement-induced (incident) pain (Portenoy et al., 1999). Pharmacological modulation with antiinflammatory drugs, opioids, and bisphosphonates frequently provides temporary relief in ameliorating tonic pain; however, new therapeutic approach are essential for management of the incident pain (Clohisy and Mantyh, 2004).

The mechanism of CIBP is currently not clear. Bone remodeling and its homeostasis are subtly maintained via bone resorption by osteoclasts and bone formation by osteoblasts. Bone destruction seems to occur due to uncontrolled osteoclastogenesis in the tumor bone microenvironment, which appears to be primarily involved in CIBP (Coleman et al., 2010). The receptor activator of NF-κB ligand (RANKL) has been suggested to be the principal trigger for osteoclast formation, function, and survival (Dougall and Chaisson, 2006). Sequestration of RANKL with OPG decreases CIBP, bone remodeling, and tumor growth within the bone. Additionally, it has been suggested that denosumab, a humanized RANKL monoclonal antibody, can prominently be able to decrease bone absorption—associated incidents such as CIBP (Fizazi et al., 2011).

Calpain-1 has been shown to degrade several substrates such as IκBα, c-jun, and c-fos, the important components involved in osteoclastogenesis (Zenz et al., 2008). Calpain-1 inhibitor can prominently decrease RANKL-induced osteoclastogenesis in murine RAW 264.7 cells (RAW 264.7 cells are a macrophage-like Abelson leukemic virus transformed cell line derived from BALB/c mice) in vitro (Lee et al., 2005; Collin-Osdoby and Osdoby, 2012). Therefore, calpain seems to confer a critical part in the regulation of osteoclastogenesis. IκBα, the inhibitory component of NF-κB, is also substrate of the calpains. The decrease in osteoclastogenesis upon calpain inhibition could be due to the attenuation of IκBα hydrolysis, which in turn leads to the inhibition of RANKL-induced NF-κB activation (Lee et al., 2005). The tartarate-resistant acid phosphatise (TRAP) positive cells (TRAP, a type of cancer cells, which attack blood and bone marrow and produce abnormal lymphocytes) in the tibia (aka the shinbone or shankbone is the larger, stronger and anterior, i.e., frontal of the two bones in the leg below the knees of the vertebrate) have been observed to be markedly attenuated upon calpain inhibition. Thus, osteoclastogenesis can be modulated by calpain inhibitors. Anti-RANKL antibody has been shown to attenuate metastatic cancer—induced bone resorption via decrease in the RANK/RANKL/OPG pathway (Zenz et al., 2008; Xu et al., 2015).

15. Castration-resistant prostate cancer

Castration-resistant prostate cancer (CRPC) is a commonly diagnosed human cancer in men, and it is known to be the second cause of cancer-related death in the Western countries. Invasion and metastasis are the primary characteristics of the malignant tumor. Even using all the currently available treatment modalities, CRPC ends up with metastasis, which is castration resistant and incurable (Amaral et al., 2012). An alteration in the prostate tumor

microenvironment and proteolysis of the extracellular matrix (ECM) by MMPs such as MMP-2 and MMP-9 mediates the process ECM degradation, which may lead to tumor invasion and metastasis (Yousef et al., 2014).

It has been shown that CAPN2 plays a critical part in the development of prostate cancer (Rios-Doria et al., 2004). Capn2 mRNA has been observed to be markedly increased in prostate carcinomas in comparison with the normal control prostate tissues (Rios-Doria et al., 2003). CRPC progression occurs via multiple steps; among which, one seems to be to reduce the ability of extracellular matrix, thereby facilitating tumor invasion and metastasis. The MMPs are known to cleave ECM proteins. Numerous researches have shown that MMP-2 and MMP-9 are involved in CRPC and exemplify as prominent prognostic components in human prostate cancer (Zhong et al., 2008). Thus, agents that abrogate MMP-2−/−9 protein levels may attenuate the migration and invasion of CRPC cells (Fiorentini et al., 2014).

16. Therapeutic intervention and calpain inhibition

In numerous human and animal models, calpain inhibition has been implicated as a prospective anticancer interposing strategy earmarking tumor cell survival, invasion, and chemotherapy resistance. Besides, calpain inhibition affects receptor-mediated signaling in many types of tumors, for example, ErbB2. ErbB2-overexpressing breast cancer cells also express other ErbB receptors and may form heterodimers. An interdependent network of ErbB pathways could be triggered upon increase in the expression of ErbB2 and that could influence tumor cell response to chemotherapeutic stress. ERBB2-expressed breast and prostate cancer cells allow cleavage of androgen receptor by calpain, thereby promoting androgen independent signaling. Notably, calpain-1 expression is associated with a worse response to trastuzumab therapy in patients with ErbB2-positive breast cancer (Kulkarni et al., 2010). Additional evidence revealed that calpain-mediated apoptosis that occurs upon cisplatin {cis-platinin (II) diamminedichloride} treatment has been observed in several cancer cell models (Storr et al., 2011; Storr et al., 2011).

In the past, anticancer effects of several peptide and nonpeptide calpain inhibitors, prepared from natural sources and also by chemical synthesis, have been tested in different models of cancer (Carragher, 2006). Peptidomimetic inhibitors are usually targeted toward the active site of calpain and are subclassified into peptidyl epoxides, peptidyl aldehydes, and peptidyl ketoamide classes (Carragher, 2006). Thus, targeting the catalytic site of calpain, for example, peptidyl inhibitors could elicit limited specificity for calpains in comparison with the other family of cysteine proteases. Majority of the peptidyl calpain inhibitors belong to the aldehyde group, for example, peptide aldehyde or their derivatives. Of note, this class of compounds are unstable, so they are clinically unattractive (Storr et al., 2011; Storr et al., 2011).

Carboxamide compounds e.g., WO2010094755 having high selectivity against calpain-I, have been developed by Abbott Pharmaceuticals. A nonpeptide compound, α-mercaptoacrylates, which targets the regulatory Ca^{2+}-binding domain of calpain, but not the active site, showed high specificity for calpains and has the potential for developing allosteric calpain inhibitors (Todd et al., 2003). Allosteric inhibitors that attach the IV and V Ca^{2+}-binding regulatory domains have been observed to be unsuccessful against some types of

calpains. Besides, quinazolinecarboxamides target calpain with high selectivity in comparison with cathepsins by interacting with a yet unknown allosteric site. Understanding the structural basis of calpain inhibition by calpastatin, the most prominent endogenous inhibitor of calpain, could enable to target calpains for developing novel cancer therapies (Hanna et al., 2008).

17. Conclusion and future direction

Capns, a group of calcium-dependent proteases, are involved in tumor progression. It is now well established that an increase in intra- and extracellular Ca^{2+} channels and transporters contributes an increase in $[Ca^{2+}]_i$ levels in metastatic malignancies. The contribution of various Ca^{2+} channels and transporters in the cell membrane and the endoplasmic reticulum membrane in different types of cancer cells are worth for investigation (Chen et al., 2016). Some studies have suggested that the frequent higher expression of Capns variably plays oncogenic roles in promoting tumor metastasis (Moretti et al., 2014), albeit some other studies have demonstrated that Capn4 activation could share apoptosis or aggressive resistance. However, to better understand the role of Capn in malignant tumor migration and invasion, it is important to determine different stimuli that activate Capns−MMPs axis and to determine their specific endogenous substrate (Chen et al., 2019).

Capn4 contributes to different types of cancers such as RCC, HCC, and NPC progression upon activating FAK-Src and subsequently modulating NF-κB signaling axis and enhances MMP-2 level. Capn4 activation, alone or in combination with P-FAK, provides a suitable prognostic biomarker, for example, for HCC patients undergoing curative resection. Therefore, Capn4 can be targeted to attenuate RCC, HCC, and NPC (Dai et al., 2014).

In human mammary MCF7 cell lines, overexpression of cyclin E demonstrated a marked increase in calpain-dependent proteolysis. Cyclin E-dependent aberrant activation of calpain-2 could be critical to confer aggressive characteristics and genomic instability. It may be the pivotal feature that confers a cellular phenotype of aggressive behavior and genomic instability in cyclin E-overexpressing MCF7 cells (Libertini et al., 2005).

In cervical carcinoma cells, calpain-6 promotes angiogenesis by inhibiting apoptosis, thereby facilitating tumor formation. Calpain-6 appears to be a promising therapeutic target for the treatment against some types of cancer. Future studies should focus the underlying mechanism by which calpain-6 inhibits caspase-3 activity during apoptosis, and also to ascertain the mechanism associated with the role calpain-6 during angiogenesis (Rho et al., 2008).

Functional characterization of endogenous calpain substrates using the latest proteomic technologies could provide useful calpain substrates. Targeting calpains with desirable substrates could help to develop specific calpain inhibitors with appropriate pharmacological specificity to proceed for drug development schemes. Besides, an in-depth examination determining the physiological consequences upon attenuating the proteolysis of calpain substrates seems crucial to avoid undesirable side effects. However, the development of selective and efficient calpain inhibitors remains a considerable challenge.

Fig. 1.4 demonstrates the contribution of calpain 2 in the dynamics of invadopodia.

Research in the recent past gained an insight into the role of calpain-2-PTB1B-cSrc signaling axis for invadopodia formation in breast cancer cells. This appears to be an

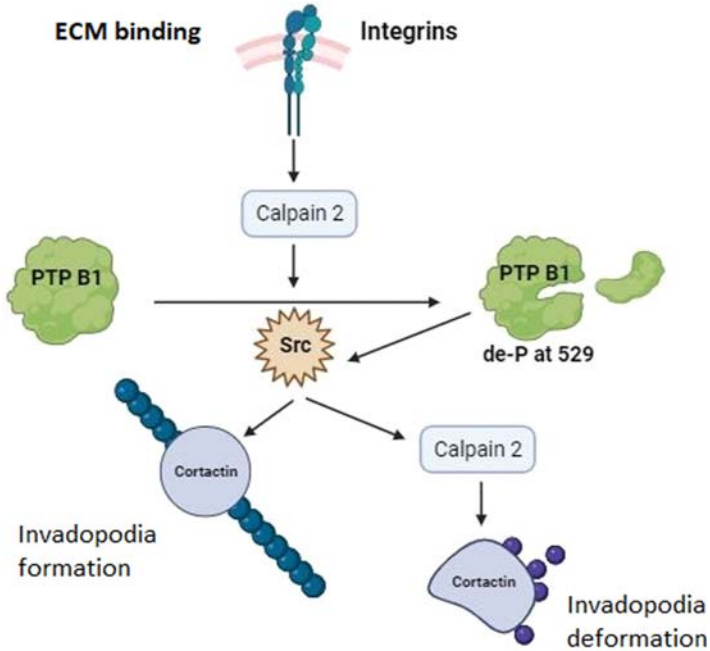

FIGURE 1.4 This figure demonstrates the contribution of calpain 2 in the dynamics of invadopodia. Calpain activity is increased by integrin interaction and/or EGF receptor activation. PTP1B is cleaved by calpain, which increases phosphatase activity. By removing the inhibitory phosphate from Src's Y529, PTP1B activates Src kinase activity and starts the development of invadopodia. By controlling the disintegration of cortactin from invadopodia, calpain 2 also acts in a downstream manner from Src kinase activity.

important aspect for regulation of calpain-mediated proteolysis that function in concert with targeted phosphorylation/dephosphorylation episode upon modulating cellular signaling pathway(s) via Src. Calpains play critical roles both upstream and downstream of Src-tyrosine kinases by acting on specific substrates, which regulate the generation and turnover of invadopodia. Conceivably, calpain effects are context dependent, because calpain-2 is not necessary for invadopodia formation in breast cancer cells transformed with v-Src. Moreover, calpains target substrates such as talin, which could regulate the effects of calpain effects on invadopodia dynamics. Future studies will conceivably provide better insight into the role of this pathway in regulating invadopodia generation and associated dynamics on matrix-degrading and breast cancer cell and metastasis (Cortesio et al., 2008).

Calpain-1 imparts an important prognostic information in OSCC and provides notable information about the malignant progression of OSCC cells. However, nonselective patients with OSCC require investigation to determine whether the increase in calpain-1 expression could be of clinical relevance. Additional research is needed to better understand the function and mechanism of calpain-1 and that could give an insight into the target for OSCC (Ma et al., 2017).

The calpain–calpastatin system plays critical roles at the developmental stages in different tissues. The proteolysis of selective calpain substrates could have multiple roles in several pathological processes, especially during cancer progression (Kar et al., 2008). Thus, calpains

inhibition by pharmacological agents could be a future direction in cancer therapy–related research.

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CHAPTER

2

Mitochondrial proteases in cancer development and progression

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1. Introduction

Proteases constitute a diverse and indispensable group of enzymes with a fundamental role across various biological processes. They catalyze the hydrolysis of peptide bonds in proteins, leading to their fragmentation and breakdown. Consequently, proteases regulate essential cellular functions such as protein turnover, immune response, and signal transduction (Turk et al., 2012). These enzymes fall into six main classes based on their catalytic mechanisms: aspartic, glutamic, cysteine, serine, threonine, and metalloproteases. Mechanisms controlling protease activity encompass gene expression regulation, activation of inactive forms (zymogens), inhibition by natural inhibitors targeting specific cellular compartments, and postsynthesis modifications such as glycosylation and disulfide bridge formation (López-Otín and Bond, 2008). Proteases are universally present in animals, plants, and microbes and located in different compartments in the mammalian cells that include mitochondria, lysosomes, Golgi apparatus, and endoplasmic reticulum.

Interestingly, mitochondrial proteases, localized within mitochondria, are pivotal for maintaining cell structure and functional integrity (Ahola et al., 2019). Their discovery in the mid-20th century marked a significant milestone in cellular biology. These specialized enzymes, intricately positioned within the mitochondrial matrix and inner membrane, have unveiled their crucial role in upholding cellular health. Functioning as vigilant guardians, they meticulously balance protein synthesis and degradation, thus playing a vital part in ensuring proper mitochondrial functionality.

Mitochondria, often termed as the cell's "powerhouses," are responsible for generating the majority of the cell's energy through oxidative phosphorylation. This essential process relies on the coordinated efforts of various proteins working together harmoniously (Annesley and Fisher, 2019). Mitochondrial proteases, with their intricate regulatory mechanisms, ensure the

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integrity of this critical organelle. They selectively break down damaged or misfolded proteins, preventing the accumulation of harmful aggregates that could impede mitochondrial function. Additionally, these proteases actively participate in recycling amino acids, optimizing the efficient utilization of cellular resources (Gottlieb and Bernstein, 2016). In addition to their fundamental role in cellular function, emerging research suggests that mitochondrial proteases may significantly influence cancer development and progression (Bulteau and Bayot, 2011). Mitochondrial dysfunction is commonly observed in cancer cells. Altered protease activity can lead to oxidative stress, impaired mitochondrial function and changes in cellular metabolism, all of which contribute to the initiation and growth of cancer (Park et al., 2020). Furthermore, mitochondrial proteases play a critical role in regulating apoptosis, and the dysregulation of these proteases can cause cells to evade apoptosis, leading to the survival and proliferation of cancer cells (Bulteau and Bayot, 2011).

Wide-ranging research has been conducted to explore the contributions of diverse proteases in the initiation, progression, and development of cancer. These enzymes play a critical role in controlling cellular processes such as cell growth, programmed cell death, and tissue invasion. They are implicated at various stages of cancer: contributing to the start of cancer by disrupting proteins that regulate growth due to genetic mutations, and aiding in cancer development and progression by breaking down the extracellular matrix to facilitate invasion, metastasis, angiogenesis, and immune evasion. Interestingly, mitochondrial proteases, which are responsible for maintaining protein integrity to ensure cellular survival, have not been as thoroughly investigated in the context of cancer research. This area presents an intriguing avenue for further exploration alongside the established role of other proteases. Degradome, the repertoire of various proteases, provides different insights into the functional dysregulation of cancer cells. Recent findings have suggested that degradome signature from breast cancer can be used to predict the prognosis and stratification risk of patients for the treatment. To fine-tune this approach, more studies are needed to elucidate the regulatory mechanism of degradome-related genes, which also contains genes from mitochondrial degradome.

Mitochondrial degradome consists of different mitochondrial components that are grouped into different catalytic classes. Depending on the function and location, mitochondrial proteases are subdivided into intrinsic mitoproteases, pseudo-mitoproteases, and transient mitoproteases (Quirós et al., 2015). This chapter discusses briefly the function of mitoproteases and summarizes the recent advances of this group of enzymes in the development, progression, and metastasis of cancer cell.

2. A short overview of mitochondrial proteases

Mitochondrial proteases are a set of enzymes that are present inside and outside the organelle to regulate the proteostasis to maintain the quality and function (Baker et al., 2011). Among the three types of mitochondrial proteases, displayed in Fig. 2.1, intrinsic mitoproteases are predominantly present inside the mitochondria to carry out the functional activity (Ahola et al., 2019). Few mitoproteases can shuttle between the mitochondria and cytosol to mediate additional proteolytic activity. Due to mutations, few mitoproteases inside the

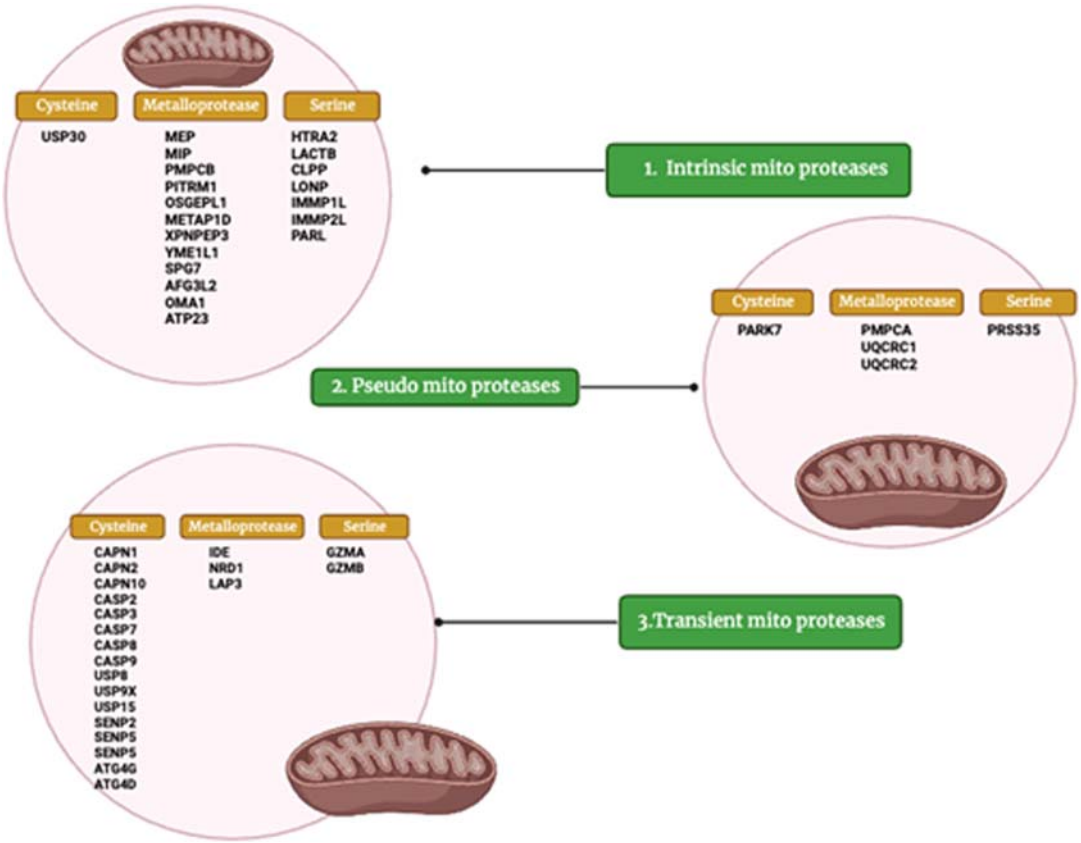

FIGURE 2.1 Types of mitochondrial proteases. A group of enzymes known as mitochondrial proteases are found both within and outside of the organelle and control proteostasis to preserve its quality and functionality. Intrinsic mitoproteases, one of the three types of mitochondrial proteases, perform the functional activity mostly inside the mitochondria. A few mitoproteases inside the organelle may undergo mutations that cause them to lose their catalytic activity, and these are known as pseudo-mitoproteases. Under some conditions, such as stress, the wandering or transient mitoproteases exhibit extra proteolytic activity after translocating into the mitochondria (Lebeau et al., 2018). These mitoproteases are further divided into three types such as cysteine, metalloproteases, and serine mitoproteases.

organelle may become catalytically inactive and are termed as pseudo mitoproteases (Reynolds and Fischer, 2015). Despite its inactivity, many of the pseudo proteases were identified to play a critical role in the cell, where they act as chaperones and may have new functions (Zhou et al., 2019). The roaming or transient mitoproteases exert additional proteolytic activities after translocated into the mitochondria under certain circumstances such as stress (Ahola et al., 2019). The intrinsic mitoproteases are further subdivided into processing peptidases (that cleaves signal sequences from mitochondrial proteins), ATP (adenosine triphosphate) dependent peptidases, oligopeptidases, and other mitochondrial peptidases (Deshwal et al., 2020). The proteases such as ATP23, IMP (inner membrane protease), MIP

(mitochondrial intermediate peptidase), OMA1, PARL (presenilins-associated rhomboid-like protein), and PMPCB (peptidase, mitochondrial processing subunit beta), XPNPEP3B are few examples of processing peptidases, while ATP-dependent peptidases include m-AAA, i-AAA, LonP, and ClpXP. Mitochondrial processing peptides mediate the import of nuclear-encoded newly synthesized proteins for the mitochondrial function (Kunová et al., 2022).

Mitochondrial proteases are divided into three different types, namely, intrinsic mitoproteases, pseudo-mitoproteases, and transient mitoproteases.

ATP-dependent proteases, situated within distinct mitochondrial compartments, play a crucial role in governing mitochondrial function through the maintenance of protein quality. Within the core protease system, the mitochondrial matrix houses Lon P, ClpXp, and m-AAA, with i-AAA anchored in the inner mitochondrial membrane (Matsushima and Kaguni, 2012). Mitochondrial ClpP, a 277-amino-acid-long serine protease, forms a stable heptamer ring with the ATP-dependent unfoldase ClpX (ClpXP) (Kang et al., 2005). Proteolytic activity of ClpXP maintains the integrity of mitochondrial respirasome by initiating the destruction of damaged and misfolded mitochondrial protein subunits (Al-Furoukh et al., 2015). Lon protease, a 959 residue-long serine protease, forms a homohexameric ring that complexes with ClpXP to exert chaperone-like activity with the help of m-AAA protease (Lebeau et al., 2018). Lon P can mediate the clearance of misfolded, oxidative damaged and unassembled proteins (Bonet-Costa et al., 2016). Moreover, it can regulate the mtDNA copy number by selectively downregulating mitochondrial transcription factor A. m-AAA protease forms a homooligomer with AFG3L2 or heterooligomer with AFG3L2 and paraplegin and has the catalytic subunit exposed toward matrix site and is localized in the inner mitochondrial membrane. This protein maintains the mitochondrial protein synthesis, network integrity, and the mitochondrial respiration along with other proteases by regulating mitochondrial protein quality and degradation (Opalińska and Jańska, 2018).

i-AAA is a hexameric membrane metalloprotease positioned in the inner mitochondrial membrane, with its catalytic site oriented toward the intermitochondrial space (IMS). i-AAA is responsible for the proteolytic removal of misfolded proteins within both the intermitochondrial space (IMS) and the inner mitochondrial membrane (IMM) (Coyne and Chen, 2019). Upon encountering stress signals such as membrane potential loss, heightened reactive oxygen species (ROS), and reduced mitochondrial DNA (mtDNA), i-AAA protease oversees the targeted complete or partial proteolysis of specific proteins, including OPA1 (optic atrophy 1), TIMM17A (translocase of inner mitochondrial membrane 17A), and ROMO1 (reactive oxygen species modulator 1) (Sprenger et al., 2019). The reciprocal proteolysis in response to low ATP level that leads to membrane depolarization is also regulated by i-AAA.

Oligopeptidases are present in both the mitochondrial matrix (e.g., PITRM1) and the intermembrane space (e.g., MEP). These proteins primarily function to further degrade the proteolytic products generated by ATP-dependent proteases. Additionally, they cleave off mitochondrial signaling sequences from newly imported mitochondrial proteins (Deshwal et al., 2020).

Additional mitochondrial proteases, typically situated in the intermitochondrial space (IMS), perform specific functions. LACTB (β-lactamase-like protein) serves as a regulator of mitochondrial lipid metabolism, as documented by Keckesova et al. (2017). Meanwhile, HTRA2 (high-temperature requirement mitochondrial serine protease A2) plays a pivotal role in preserving mitochondrial cristae structure.

3. Mitochondrial protease in human diseases

Mitochondria plays a key role in deciding the life and death of a cell, as they are involved in various cell maintenance that include both adaptive and nonadaptive cellular changes, survival, and death (Snyder and Chandel, 2009). To carry out these multiple tasks, mitochondria have to undergo different structural modifications via various processes such as fission, fusion, and mitophagy (van der Bliek et al., 2013). This helps mitochondria for a better networking system within the cell and effective communication to different cell organelles. Damage or derangement of the mitochondrial dynamics and its function will turn the organelle to act as source for negative internal cue for the development of different disease pathology and its progression (Chen et al., 2023). This includes many noncommunicable diseases (NCDs) such as cancer, cardiovascular diseases, neurodegenerative diseases, diabetes mellitus, brain injury, renal diseases, and chronic obstructive pulmonary disease. Thus, the proportion of heathy mitochondria in a cell can prevent or arrest the progression of many NCDs that includes cancer. Various mitochondrial quality control mechanisms in a cell such as mitochondrial fission, fusion, biogenesis, and mitophagy are responsible for the maintenance of healthy mitochondria (Picca et al., 2018). For instance, the loss of metalloprotease PITRM1, also known as PreP, results in malfunctions in oxidative phosphorylation (OXPHOS), modifies the expression of genes that mark stress responses within the nucleus, and brings about a buildup of presequence peptides (Lebeau et al., 2018). This buildup sets off a mechanism that inhibits the mitochondrial processing of peptidase (MPP) through feedback. In a similar way, the absence of functional mitochondrial peptidase, PITRM1, creates a state of stress due to improper protein digestion, contributing to conditions reminiscent of Alzheimer's disease in humans (Pérez et al., 2021). Thus, the coordination of all these events is mediated by a battery of proteins, and these proteins are controlled by a set of mitochondrial-specific proteases.

The major challenges faced by the mitochondria to maintain its functional proteome are from three major sources: (1) 99% of mitochondrial proteins are encoded by nuclear genome, where the majority of proteins are formed in the cytosol and are transported into mitochondria, and to maintain their structures inside the organelle is challenging; (2) the key protein complexes such as those present in different redox and nonredox protein complexes of respirosome are made of different subunits and each one may be under the control of either nuclear or mitochondrial genome; and (3) the generation of free radical during the respiration from complex I and III, protection from ROS attack on the protein, lipid, and DNA present in the mitochondria will be another challenge (Fox, 2012). Fig. 2.2 summarizes different diseases that develop due to the mitochondrial protease dysfunction.

Mitochondrial proteases control all functions of mitochondria, and its malfunction can lead to many diseases.

4. Mitochondrial proteases and cancer prevalence

Even though there exists a slight drop in cancer prevalence in developed countries, the frequency of new cases keeps on increasing especially in poor countries. Among the different

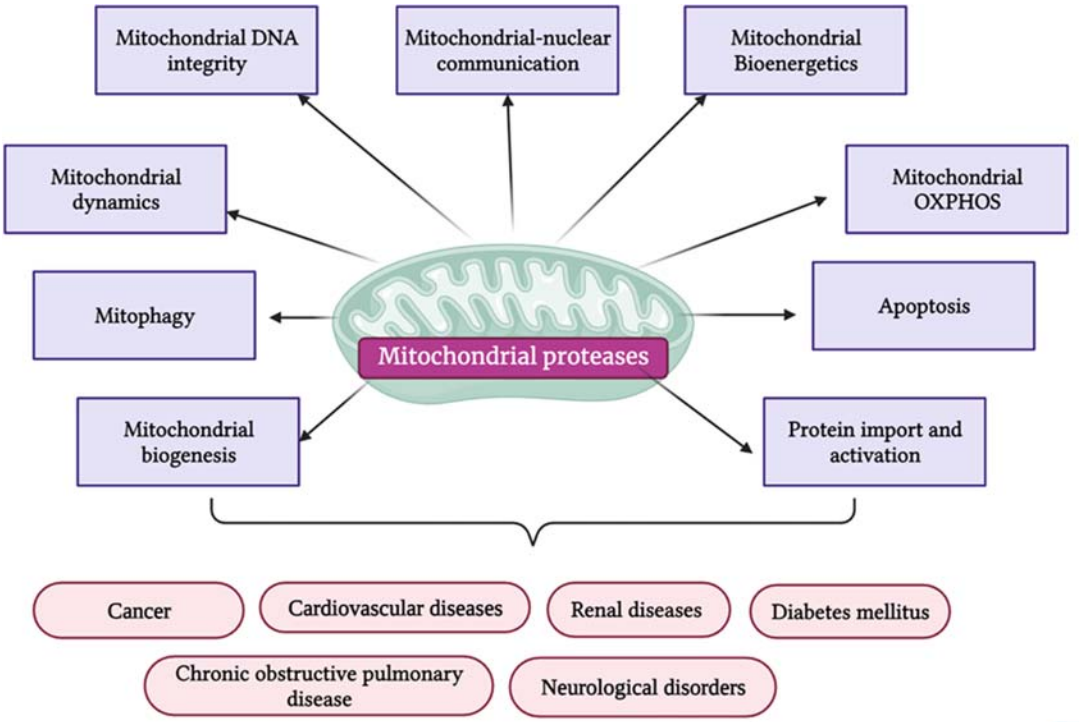

FIGURE 2.2 Comprehensive regulation of mitochondrial functions by mitochondrial proteases. Mitochondrial proteases constitute a complex ensemble of enzymes responsible for the regulation and upkeep of mitochondrial functions. They exert control over nearly all aspects of mitochondria, encompassing bioenergetic functions, cellular apoptosis, the import of diverse proteins, quality control mechanisms, and the interplay between mitochondria and the cell nucleus. Disruption or genetic mutations affecting these proteases can give rise to a spectrum of human ailments through multiple mechanisms. Firstly, when mitochondrial proteases become impaired or dysfunctional, it can lead to the accumulation of damaged or improperly folded proteins within the mitochondria. This, in turn, disrupts mitochondrial function and contributes to diseases associated with energy metabolism. Moreover, these proteases also play a crucial role in safeguarding the integrity of mitochondrial DNA (mtDNA). Mutations or malfunction in these proteases can trigger mutations in mtDNA, leading to mitochondrial diseases that primarily impact tissues with heightened energy requirements. Dysfunctional proteases can additionally impede the process of mitochondrial biogenesis, resulting in a decreased count of functional mitochondria and a subsequent reduction in energy production. Furthermore, the malfunction of mitochondrial proteases is linked to various neurodegenerative diseases, mitochondrial myopathies, cardiovascular disorders, metabolic irregularities, respiratory complications, and more.

cancers: lung, breast, prostate, and colon accounts the higher prevalence. The involvement of mitochondrial protease especially ATP-dependent mitochondrial proteases in the pathophysiology of cancer is well known. Previous studies have shown that mitochondrial gene mutation in cancer deteriorated the mitochondrial quality and the regulatory machinery that in turn is linked with OXPHOS and also the redox regulation of ROS. Thus, the prevalence rate increases when the tumor microenvironment is altered in support to tumor growth and progression where the modified mitochondrial role is indispensable.

The association of proteolytic enzymes with cancer is well established where they are involved in tumor growth, invasion, angiogenesis, and metastasis (Park et al., 2020). Considering the importance of mitochondrial dysfunction in cancer cell development, progression, and metastasis, deciphering the contributory role of proteases present in the organelle is critical in understanding the pathology (Boland et al., 2013). Recent studies have shown that downregulating mitochondrial protease Lon P in cervical cancer can suppress cell proliferation via mitochondrial remodeling and suppression of autophagy. Further in bladder cancer, few investigators have shown that Lon P inhibition can reduce cell proliferation by promoting apoptosis, enhanced chemosensitivity, and decreased bioenergetics (Liu et al., 2014). In pancreatic cancer, investigators have demonstrated that inhibition of Lon P1 declined the cancer cell progression via c Jun N terminal kinase pathway−mediated epithelial mesenchymal transition (Liu et al., 2019). High grade of gliomas are also reported to have increased expression of mitochondrial Lon protease expression (Di et al., 2016). Similarly, Clp XP inhibition is demonstrated to be effective in the management of human acute myeloid leukemia (Meβner et al., 2021). ClpP was also found to be involved in pancreatic ductal adenocarcinoma, where its aberrant activation can disturb mitochondrial proteome homeostasis (Wang et al., 2022). Similarly, mutations in i-AAA and m-AAA have been associated with various mitochondrial disorders, such as spinocerebellar ataxia with axonal neuropathy (SCAN1), spinocerebellar ataxia type 28 (SCA28), and also in cancer development and progression.

5. Mitochondrial proteases and cancer development

Cells normally utilize proteolysis to control the events involved in cell cycle that mediates the duplication of cells. Two distinct ubiquitin-conjugating proteosomes are involved in regulating cell cycle process by degrading CDK inhibitor and anaphase inhibitor and mitotic cyclins (Zou and Lin, 2021). Ubiquitin proteasome systems are also located inside the mitochondria (Kodroń et al., 2021). Previous studies have shown that Lon P1 protease promotes proteasome inhibitor resistance in multiple myeloma and its overexpression can reduce the efficacy of proteasome inhibitors (Maneix et al., 2021). The activity of proteases in the mitochondria maintains the quality of the organelle that could support the energetic demand imposed by proliferation (Ng et al., 2021). The energy-consuming activity associated with cell cycle is controlled by checkpoints. Thus, the integrity of mitochondrial machinery controlled by a set of proteases inside the organelles, when nonfunctional or altered, ultimately leads to the loss of cell cycle regulation that controls the proliferation rate (Arciuch et al., 2012). The energy derived from the mitochondria will be used to coordinate the G1 phase events and S/G2/M phase events. Since mitochondrial proteases control the organelle-specific changes in their morphology, dynamics, and during cell cycle, these changes allow the effective segregation of the organelle and mtDNA into daughter cells, the defect or mutations in these proteases promote the cancer pathology (Arciuch et al., 2012). This emphasizes the significant role of metabolic alterations in the pathogenesis of different cancers along with the cell cycle regulators (tumor suppressor proteins) and oncogenic proteins. The mechanism of mitochondrial proteases in the development of cancer is displayed in Fig. 2.3.

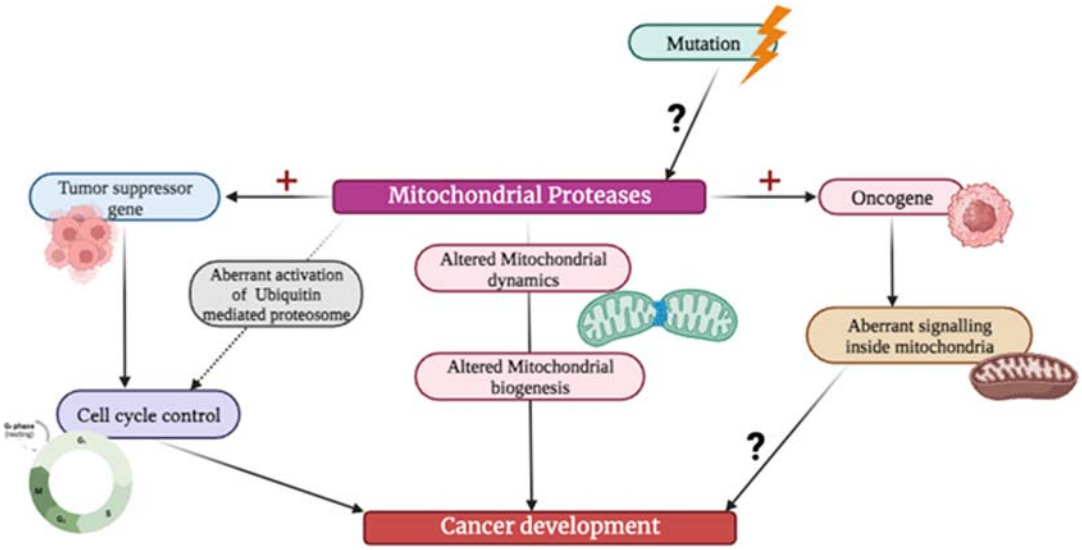

FIGURE 2.3 Mitochondrial proteases in cancer development. Cells utilize proteolysis to control the cell cycle, ensuring proper cell duplication. The cell cycle is regulated by two distinct ubiquitin-conjugating proteasomes, which degrade CDK inhibitors, anaphase inhibitors, and mitotic cyclins. Mitochondrial proteases play a crucial role in maintaining organelle quality, essential for meeting energy demands during cell proliferation. Dysfunction in these proteases disrupts cell cycle regulation and mitochondrial energy utilization, contributing to cancer development. Similarly, the dysregulated mitochondrial proteases may fail to counter oncogenic stress, resulting in altered mitochondrial dynamics, biogenesis, and supporting tumorigenic growth.

Cells regulate the cell cycle via proteolysis, relying on two ubiquitin-conjugating proteasomes to degrade CDK inhibitors, anaphase inhibitors, and mitotic cyclins. Mitochondrial proteases maintain organelle quality, and its dysfunction can lead to the development of cancer.

The oncogenic transcription factor c-MYC is known to target over 400 mitochondrial genes, and its gain or loss of function can either increase or reduce mitochondrial mass (Stine et al., 2015). Few studies have shown that targeting c-MYC is effective in the management of cancer patients (Duffy et al., 2021). Similarly, oncogenic pathways that influence the mitochondrial biogenesis and mitophagy where mitochondria act as a physical platform for protein—protein signaling interactions and regulate intracellular signaling mediators are indirectly associated with protease activity inside the organelle and thus promote cancer development. Mitochondrial proteases that act as defensive agent against the cellular oncogenic stress may fail in the presence of mutated oncogenes (O'Malley et al., 2020). This in turn mediates the aberrant downstream effector signaling pathways that regulate the mitochondrial dynamics machinery to stimulate the changes in mitochondrial morphology and its physiologic consequences of altered mitochondrial morphology for tumorigenic growth.

6. Mitochondrial proteases and cancer progression

Cancerous cell in tissues is not easily monitored or observed due to their low concentration, and thus, it is mostly asymptomatic. The molecular and cellular events that make the

cancer progression and become symptomatic are less well understood. Breaching the tissue architecture needs dissolution of the cell to cell adhesive boundaries along with remodeling of cytoskeletal frameworks. Additionally, carcinomas generally invade their neighboring cells or tissues as a single cell upon epithelial-to-mesenchymal transition or during epithelial cluster formation (Roche, 2018). The major driving force for all these events is stemmed from the stress experienced by the tumor cells because of mutations in their oncogenes and tumor suppressor genes. This stress is normally generated from cell to cell and cell to extracellular matrix (ECM) tension, interstitial fluid pressure, compression stress, and shear stress. The transformation of nonsymptomatic cancer cell to symptomatic one that ultimately becomes malignant tumor depends on the expansion of cancerous cells in their local environment (Quail and Joyce, 2013). Often the disturbance of proteases metabolism that includes the production, activation, and inhibition decides the tumor cell invasion and dissemination.

Interestingly, proteases can mediate the progression of cancer, including migration and metastasis, and are also involved in the suppression of tumor growth (Mason and Joyce, 2011). According to human degradome data, out of 569 proteases, 194 are metalloproteases, 176 are serine proteases, 150 are cysteine proteases, 28 are threonine proteases, and 21 are aspartate proteases (Choi et al., 2012). Matrix metalloproteases are known to promote tumor growth by degrading matrix barriers and by enhancing angiogenesis (Quintero-Fabián et al., 2019). Growing evidence in the literature showed that cell surface−anchored serine proteases are deregulated in cancer and are thus involved in both tumor formation and metastasis (Martin and List, 2019). Cysteine proteases are also involved in cancer development and progression by involving in the degradation of ECM and promote the invasion and metastasis process (Rudzińska et al., 2019). Threonine proteases that act as proteasomes are also involved in cancer development and progression (Vizovisek et al., 2021). Various studies have implicated the role of cathepsins in the regulation of tumor angiogenesis (McDowell et al., 2020).

Mitochondrial support in tumorigenesis is involved in different stages of cancer that includes initiation, growth, survival, and metastasis (Vyas et al., 2016). Oncometabolite production by the mutated mitochondrial genes along with increased oxidative stress and mitochondrial signaling can not only initiate the cancer development but also provide driving force for the progression by changing the tumor environment. Many studies in the past showed that mutations in mtDNA are present in many tumor cells where the binding site of Lon protease and TFAM in the D loop region are the hot spot, thereby affecting the bioenergetics efficiency (Wagner et al., 2022). Reprograming the metabolism of mitochondria by the tumor environment helps the growth of cancer cells by modulating the mitochondrial biogenesis, dynamics, regulating redox signaling, and oxidative stress (Falone et al., 2019). Rewiring the mitochondrial proteome in response to changed tumor environment is essential for the metabolic reprogramming, which is mediated by different mitochondrial proteases. i-AAA proteases that control biogenesis of mitochondrial protein and lipids, fusion and fission events, and metabolic profiling are reported to be altered in cancer cells (Opalińska and Jańska, 2018). Similarly, ATP-dependent Lon proteases that control tumor biogenesis by reprogramming mitochondrial bioenergetics activity helps in the progression of cancerous cells (Quirós et al., 2014). The role of mitochondrial proteases in cancer progression is summarized in Fig. 2.4.

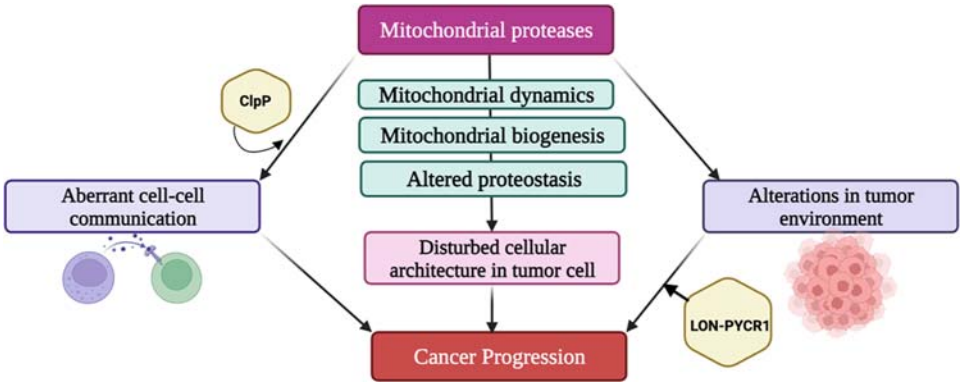

FIGURE 2.4 Mitochondrial proteases in cancer progression. The progression of cancer involves the breakdown of cell-to-cell adhesive barriers and the reshaping of cytoskeletal structures, leading to carcinoma invasion either as individual cells or clusters. These processes are fueled by stress induced by mutations in oncogenes and tumor suppressor genes. Disturbances in protease activity, influencing production, activation, and inhibition, have a notable impact on tumor cell invasion and dissemination. Proteases play pivotal roles in cancer by facilitating migration, metastasis, and the inhibition of tumor growth. Mitochondrial support plays a critical role in tumorigenesis, with mutated mitochondrial genes, oxidative stress, and mitochondrial signaling driving the development and progression of cancer. The adaptation of the mitochondrial proteome in response to the tumor environment is governed by a variety of mitochondrial proteases, which affects the metabolic changes happening within the cell.

Cancer progression involves disrupting cell adhesion and cytoskeletal structures, enabling carcinoma invasion driven by oncogene and tumor suppressor mutations. Disturbances in mitochondrial proteases impact invasion, migration, and metastasis. Mitochondrial dynamics and proteases govern tumorigenesis and metabolic changes.

7. Mitochondrial proteases and cancer metastasis

What we know from decades that the mitochondrial role is critical in the transformation of normal cell to cancerous cells to support the outcome of cardinal mutations in protooncogenes and tumor suppressor genes that eventually leads to cancer. In addition to the metabolic role of mitochondria, the alterations in mitochondrial genomes significantly contribute to the cancer development. In fact, cancer cells exploit mitochondria for metastatic competence. This involves different mitochondrial events, namely, bioenergetics, redox balance, dynamics, and phosphorylation of signaling kinases. Tumor environments are not favorable to mitochondrial health and are characterized with an environment having erratic oxygen concentration, accumulation of ROS, and vulnerable mitochondrial proteome.

Motility and invasion, microenvironment modulation, plasticity, and colonization are the hallmarks of cancer metastasis, where the mitochondrial biochemical milieu in the cancer cell and stromal cell and its interactions are crucial for metastasis (Scheid et al., 2021). The metabolic outputs of mitochondria, either from oxidative phosphorylation or glycolysis, influence the invasion and motility of cancer cells especially its epithelial-to-mesenchymal transition.

Similarly, mitochondrial role in tumor microenvironment modulation is envisaged with its ability to cross-talk with cancer cells and microenvironment (Scheid et al., 2021). The major components of this changed microenvironment consists of endothelial cells, fibroblasts, immune cells, tissue resident epithelial cells, and microbiome. Through secretion of extracellular vesicles and tunneling nanotubes containing mitochondria-derived molecules, tumor cells can communicate and influence other cells in its microenvironment (Roehlecke and Schmidt, 2020). Another hallmark of metastasis, plasticity ensures that the initial engagement of invasive and migratory genes is followed by the proliferative genes reexpression upon reaching the metastatic site, to facilitate outgrowth (Yuan et al., 2019). Mitochondrial role in this process mainly is centered on its ability to regulate the epigenetic level of gene expression resulted from the changing tumor microenvironments, hypoxia, and immune infiltration (Scheid et al., 2021). This helps the smooth transition of epithelial-to-mesenchymal and vice versa. The fourth hallmark of metastasis, colonization requires healthy tumor cells and responsive microenvironment and also depends on the balance between proliferation and apoptosis, and mitochondrial biogenesis and mitophagy.

Consistent with the essential roles of mitochondrial protease in maintaining OXPHOS function, expression of ClpP, Lon P, m-AAA, and i-AAA has correlated with poor prognosis and lower metastasis-free survival in cancer patients especially with acute myeloid leukemia, breast adenocarcinoma, uveal melanoma, and lung adenocarcinoma.

8. Mitochondrial proteases and cancer stem cells

Mitochondrial metabolism is necessary for the determination of stem cell fate and function where its signaling ability coordinates the whole process via supporting the differential metabolic needs of stem, progenitor, or differentiated cells (Chakrabarty and Chandel, 2021). Mitochondrial role in regulating stem cell fate is associated with generated ROS, TCA cycle metabolites, mitochondrial dynamics, and the NAD^+/NADH ratio. Low levels of endogenous ROS are reported in pluripotent stem cells (PSCs) and embryonic stem cells (ESCs) to maintain its genome integrity (Bigarella et al., 2014). The differentiation of PSC or ESC into different lineages needs a moderate increase in ROS levels. Moreover, ROS in mitochondria are found to be the regulator of quiescence, activation, proliferation, differentiation, and exhaustion of adult stem cells (Zhou et al., 2019). TCA cycle metabolites such as acetyl-CoA, α-keto glutarate, succinate, and fumarate alter the epigenetic modifications of genes via methylation and acetylation to change the gene expression that is necessary to determine stem cell fate (Martínez-Reyes and Chandel, 2020). Differentiated and nondifferentiated stem cell exhibited different morphology of mitochondria, where fragmented, globular mitochondria are predominant in stem cells, whereas their differentiated counterparts contain elongated, tubular mitochondria (Seo et al., 2018). Similarly, previous studies have reported that mitochondrial fission is necessary for the reprogramming of somatic cells into induced pluripotent stem cells (iPSCs).

Mitochondria also play key roles in cancer stem cells (CSCs) metabolism (therapy resistance subpopulation of cancer cells), maintenance of stemness, and differentiation that regulate the cancer cell progression (Zheng et al., 2023). CSCs with tumor-initiating capacity reside in specialized tumor microenvironment, where proteases regulate CSC activities

such as extracellular matrix remodeling, self-renewal capacity, resistance to radiation therapy, chemotherapy, and cell motility (Plaks et al., 2015).

However, the normal stem cell microenvironment and cancer stem cell niche are different despite the common characteristic feature as stemness. Hence the role of mitochondria in stem cell regulation in normal and cancerous cell also may differ. In gliomas, the higher sensitivity of proteasome inhibitor toward CSC makes it a promising therapeutic drug (Ji et al., 2010). Proteasome inhibitors are one of the standard therapeutic drugs in the combination therapy for cancer especially multiple myeloma and gliomas. Studies have shown that targeting only proteasome is not very effective, but when both cytosolic proteasome and mitochondrial proteases such as LonP1 are targeted, the beneficial effect will be enhanced for the treatment of multiple myeloma (Maneix et al., 2021). Proteasome activities are low in CSC marker, and the mitochondrial proteases are believed to counterbalance protein degradation in cancer cells treated with proteasome inhibitors (Maneix et al., 2021). The agonist of mitochondrial Clp proteases such as ONC201 can inhibit breast cancer cell proliferation and its CSC function via downregulating mevalonate pathway, YAP, Myc, and the HIF pathway (Greer et al., 2022). Intriguingly, in leukemia stem cells (LSCs), both inhibition and activation of Clp protease lead to decreased OXPHOS, indicating the need of balance in protease activity to support OXPHOS in LSCs (Kreitz et al., 2019).

9. The emerging role of mitochondrial protease in cancer therapy

Cancer cells are reported to experience mitochondrial proteostatic stress that enables the acquisition of aggressive phenotype to cancer cells that help them to be chemoresistant and metastatic. Hence, many investigators utilized the approach to target mitochondria to kill cancer cell via activating apoptosis, prevent or slow down metastatic progression. Different research groups have shown that the cancer cell death can be mediated by increasing the mitochondrial calcium flux into the organelle or by inducing the sustained opening of mitochondrial membrane permeability pore (Bonora and Pinton, 2014). Mito serine protease Omi/HtrA2 is known to be involved in both apoptosis and caspase independent cell death (Hegde et al., 2002). Once this protein is released into the cytosol, it triggers cytochrome C dependent caspase activation by negating the inhibitory effect of apoptotic protein. By using triphenyl phosphonium cations, few investigators targeted mitochondria to enhance direct killing of precancerous and dysplastic cells for effective chemoprevention (Zielonka et al., 2017). In addition, the prognostic potential of mitochondrial ribosome gene transcript in predicting distant metastasis in ER (+) breast cancer patients emphasizes the utility of proteases for developing prognostic biomarkers (Bao et al., 2022). Accordingly, Clp protease can be a potential biomarker in cancer treatment as they are involved in the control of mitochondrial ribosomal quality (Nouri et al., 2020). But existing studies are limited to ascertain the comparative potential of different classes of mitoriboscins such as (1) mitoribocyclines; (2) mitoribomycins; (3) mitoribosporins; and (4) mitoribofloxins to act as either prognostic or diagnostic markers (Ozsvari et al., 2017).

According to the recent studies, in ovarian cancer, ClpP levels hold diagnostic promise for distinguishing tumor types. Even though it is not directly associated with tumor type like

oncogene or tumor suppressor genes, its association to cancer research is linked to mitochondrial role in tumor development and progression. Few researchers have shown distinct impact of mitochondrial dysfunction in different types of cancer such as breast cancer, colorectal cancer, and other malignancies. Similarly, Lon protease upregulation is effective in the prognosis of adverse breast cancer, but few other studies demonstrated that its high level is a poor prognostic marker in human colorectal cancer. In fact, by using gain of function and loss of function of Lon P gene experiments, investigations have emphasized the critical role of Lon protease in cancer development (Quirós et al., 2014). These data from the literature effectively suggest that the biomarker potential of mitochondrial proteases depends on the complexity of tumor environment.

Exploiting mitochondrial proteases for treatment encompasses UCHL1-mediated oncoprotein degradation in multiple myeloma (Hussain et al., 2015) and selective inhibition sch as aclacinomycin A in leukemia (Schütte et al., 1983). Mitochondrial AAA proteases, specifically YME1L1, hold significant prognostic implications in ovarian cancer, as elevated expression of YME1L1 has been linked to poor prognosis and advanced disease stages in ovarian cancer patients (Liao et al., 2022). Furthermore, YME1L1 levels could potentially serve as a valuable biomarker for assessing the aggressiveness of ovarian cancer. Similarly, the downregulation of mitofusin-2 (MFN2), a protein involved in mitochondrial fusion, emerges as a predictive factor for the progression of nonmuscle invasive bladder cancer (NMIBC), where different forms of mitochondrial fusion genes are processed via mitochondrial proteases such as YME1L1. Reduced MFN2 expression is correlated with higher tumor grades and increased recurrence rates in NMIBC patients, highlighting its potential as a diagnostic indicator. Precision medicine approaches could tailor treatments based on individual mitochondrial protease profiles, paving the way for more personalized and potent therapies. Targeting mitochondrial proteases might also be beneficial in overcoming drug resistance in cancer cells and providing an alternative to restoring sensitivity to standard treatments.

10. Chemical modulators of mitochondrial proteases in cancer therapy

One promising area of cancer research involves the use of chemical modulators, which are compounds capable of selectively influencing specific cellular processes and molecular pathways crucial for tumor growth and progression. These chemical modulators include inhibitors that disrupt key pathways or protein functions essential for cancer cell survival, as well as activators that enhance the activity of pathways promoting cancer cell death. In recent years, significant progress has been made in identifying and developing chemical modulators targeting various cellular components and signaling pathways implicated in cancer.

Acyldepsipeptidase (ADEPs), activator of ClpP, is reported to inhibit renal cancer cell growth by modulating G1 phase of cell cycle regulator (Xu et al., 2013). It was also found to possess cytotoxicity in breast cancers. The imipridone ONC201, the analog of ADEP antibiotics, is currently in cancer clinical trials, identified to be effective against a broad range of tumor cells but not normal cells (Jacques et al., 2020). ADEPs that bind to human ClpP induce caspase-dependent apoptosis, inhibit mitochondrial respiration, and inflict mitochondrial fragmentation in HEK293 cells. These chemical inhibitors that include ONC201

and other imipridone degrade the mitochondrial protein resulting in loss of mitochondrial integrity (Jacques et al., 2020). Importantly, these agonists of ClpP can activate ClpP in the absence of ClpX. Rhodanine derivatives also modulate ClpP activity by interfering with its proteolytic function and thereby selectively killing cancer cells (Szczepański et al., 2022). On the other hand, certain compounds have been identified as activators of ClpP, capable of enhancing its proteolytic activity. In addition to chemical-based activation of ClpP, few investigators have shown the potential of active ClpP mutant developed by engineering a point mutation (Y118A) in human ClpP as an anticancer effect. These ClpP activators have the potential to induce proteotoxic stress in cancer cells, leading to cell death and inhibiting tumor growth.

Although challenges persist, the progress in developing chemical modulators for proteases presents a promising path toward innovative and targeted cancer treatments. Ongoing research in this field has the potential to bring about a significant transformation in cancer therapy approaches, offering novel therapeutic options for patients in the coming years.

11. Conclusion and future perspectives

One of the hallmarks of cancer development involves the deregulation of mitochondrial function, which is responsible for the altered cellular metabolism and bioenergetics, evasion of apoptosis, and elevated oxidative stress in cancer cells. Given that these changes are directly or indirectly controlled by mitochondrial protein quality that are governed by mitochondrial proteases, the critical role played by these proteases in the cancer development, progression, and metastasis cannot be ruled out. In fact, the mitochondrial proteases such as Lon P, ClpP, i-AAA, and m-AAA are altered in its function and expression in various cancerous conditions, especially in breast and neurons. Overexpression of Lon P in several tumors reported to develop aggressive phenotype of cancer. Similarly, other proteases such as i-AAA protease YME1L, Clp X, and serine protease HTRA2 have been associated with several cancers, including ovarian cancer, breast cancer, lung cancer, or hepatocellular carcinoma. Mitochondrial proteases such as LonP1, ClpXP, and others are needed to ameliorate mitochondrial stress and promote cancer cell survival and metastasis. Aberrant expression of these proteases not only helps in the development of cancer but also promotes progression and metastasis. Since cellular stress stimuli and metabolic alterations in cancer modulate the activity of protease in the mitochondria, comprehensive role of mitochondrial protease in different cancer types and stages of cancer will provide an insight into the disease pathophysiological events/mechanism. This will open a new window for a possible targeted cancer therapy.

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Declaration

No section, including figures and tables, in this chapter is obtained from any published article.

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CHAPTER

3

Proteases in head and neck cancer

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1. Introduction

Proteases (also known as peptidases, proteolytic enzymes, or proteinases) are involved in many processes associated with the development and progression of cancer, most notably extracellular matrix (ECM) degradation. The ECM is the structural support of the extracellular space, being one of the components of the tumor microenvironment along with stromal cells. There are around 500 proteases that correspond to 2% of human genes. There are several types of proteases, namely metallo, cysteine, serine, aspartic, and threonine proteases as shown in Fig. 3.1 (Rakash, 2012; Soond et al., 2019; Vizovisek et al., 2021). With the growing knowledge of the various roles that proteases play in cancer progression, their impact on diagnosis, prognosis, and response to therapy has been increasingly studied. Detecting tumors at an early stage of the disease and predicting prognosis are fundamental to the clinical management of patients. Tumors diagnosed at an early stage have better survival outcomes. On the other hand, predicting prognosis makes it possible to adjust therapies and patient monitoring. There are still few diagnostic and/or prognostic biomarkers of cancer that have been implemented in clinical practice. There are several proteases with the potential to become diagnostic and/or prognostic biomarkers. Here, we reviewed the proteases with the greatest potential to be translated into clinical practice as cancer biomarkers. The genetic alterations of proteases with an impact on diagnosis and prognosis were also described (Dudani et al., 2018).

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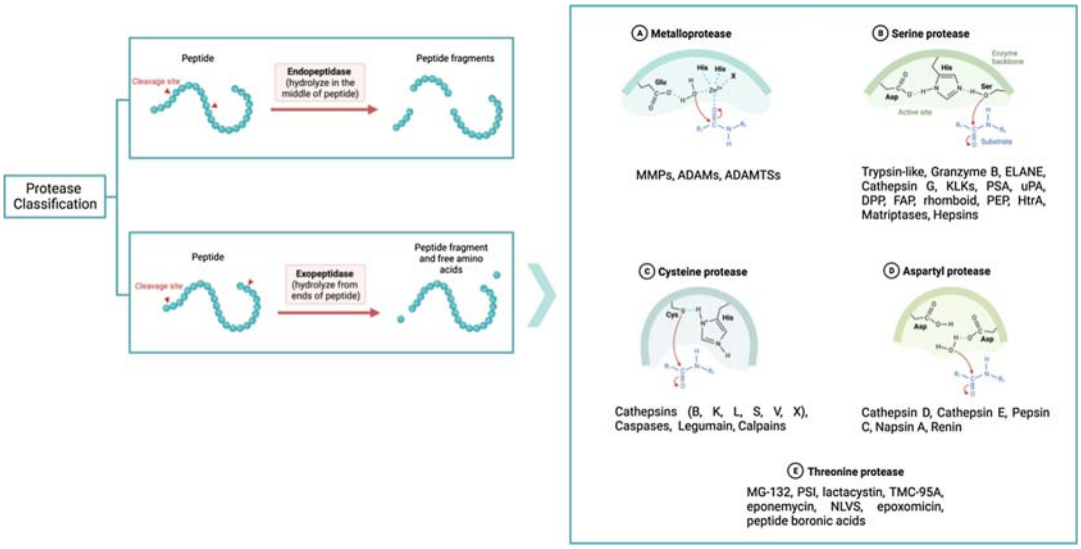

FIGURE 3.1 Protease classification. Proteases can be classified into endopeptidases and exopeptidases according to their catalytic activity. Exopeptidases can be classified as serine, cysteine, aspartic, metallo, and threonine proteases. *Created in BioRender.*

2. Metalloproteases

Metalloproteases or metalloproteinases are enzymes whose catalytic mechanism involves binding predominantly to Zn^{2+}. They are enzymes that play a very important role in regulating the remodeling of ECM. The main groups of metalloproteases are matrix metalloproteases (MMPs), disintegrin and metalloproteases (ADAMs), and disintegrin and metalloproteinase with thrombospondin motifs (ADAMTSs) as shown in Table 3.1 (Alaseem et al., 2019). The activity of metalloproteases is regulated by the tissue inhibitor of metalloproteinases (TIMP) (Jackson et al., 2017). The secreted MMPs are subdivided into collagenases (MMP1, MMP8, MMP13), stromelysins (MMP3, MMP10, MMP11, MMP12), gelatinases (MMP2, MMP9), and matrilysins (MMP7, MMP26) (Klein and Bischoff, 2011). Membrane-anchored MMPs can be classified into type I transmembrane MMPs (MMP14, MMP15, MMP16, MMP24), glycosylphosphatidylinositol (GPI)-anchored MMPs (MMP17, MMP25) and type II transmembrane MMPs (MMP23) (Rakash, 2012). Collagenases are responsible for digesting various substrates. The substrates of collagenases are collagen types I, II, III, V, VII, VIII, X, and IL-1β, MMP2, MMP9, and fibronectin. Gelatinases have a high specificity for denatured collagen. MMP2 and MMP9 digest gelatin using fibronectin type II repeats that bind to the molecules to be degraded. It is also responsible for digesting elastin, laminin, and various types of collagens. Stromelysins are stromal metalloproteases. Structurally, they are very similar to the previous ones, but they don't cleave interstitial collagens. Matrilysins are the smallest MMPs because they lack a hemopexin domain. They are responsible for digesting collagen type IV and glycoproteins. Membrane-type MMPs are activated inside the cell and exert their action on the surface. Apart from MMP17, they can activate proMMP2 and

TABLE 3.1 Metalloproteases characterization in terms of substrate, subcellular location, and secretion. The information related to subcellular location and secretion was extracted from Human Protein Atlas (HPA) and cansar.ai.

Metalloproteases type	Metalloproteases subtype	Protease	Main natural substrates	Subcellular location (HPA and cansar.ai)	Secretion (HPA and cansar.ai)
MMPs	Matrilysins	MMP7, 26	Collagen type IV, glycoproteins, gelatin	• Nucleoplasm • Cytosol • Vesicles	• Secreted to ECM
	Collagenases	MMP1, 8, 13	Collagen type I, II, III, V, VII, VIII, X, gelatin, fibronectin, MMP2, MMP9, IL-1β	• Vesicles (MMP1, 8)	• Secreted to ECM (MMP1, 8, 13)
	Stromelysins	MMP3, 10, 11, 18	Proteoglycans, fibronectin, laminin, elastin, gelatin, vitronectin, plasminogen, fibrinogen, fibrin, plasminogen, collagen type III, IV, V, antithrombin III, MMP1, MMP2, MMP8, MMP9, MMP13	• Cell membrane MMP10) • Cytosol (MMP10, 11) • Golgi apparatus (MMP11) • Endoplasmic reticulum (MMP18) • Vesicles (MMP3, 18)	• Secreted to ECM (MMP3, 10, 11, 18)
	Gelatinases	MMP2, 9	Collagen type I, IV, V, VII, X, gelatin, elastin, laminin	• Cytosol (MMP9) • Vesicles (MMP2)	• Secreted to ECM (MMP2) • Secreted to blood (MMP9)
	Membrane-type MMPs	MMP14, 15, 16, 17, 24, 25	Collagen type I, II, III, gelatin, elastin, laminin, fibronectin, fibrin, proMMP2, proMMP13	• Cell membrane (MMP15, 24, 25) • Cytosol (MMP14, 15, 16) • Intermediate filaments (MMP14) • Nucleoplasm (MMP15) • Vesicles (MMP16) • Nucleoli (MMP24)	• Secreted to ECM (MMP17, 24) • Secreted to blood (MMP25)
	Other MMPs	MMP12, 20, 23, 27, 28	Amelogenin, aggrecans, elastin	• Cell membrane (MMP23) • Cytosol (MMP28) • Organelles membrane (MMP27)	• Secreted to ECM (MMP12, 20, 23, 27, 28)

(*Continued*)

TABLE 3.1 Metalloproteases characterization in terms of substrate, subcellular location, and secretion. The information related to subcellular location and secretion was extracted from Human Protein Atlas (HPA) and cansar.ai.—cont'd

Metalloproteases type	Metalloproteases subtype	Protease	Main natural substrates	Subcellular location (HPA and cansar.ai)	Secretion (HPA and cansar.ai)
ADAM	Type I	ADAM1, 8, 9, 12, 13, 15, 19, 20, 21, 24, 26, 28, 30, 33, 40	• ADAM8: Amyloid precursor protein, CD23, CD30, TNF-α, L1, L-selectin • ADAM9: Amyloid precursor protein, c-kit ligand, collagen XVII, DLL1, EGF, HB-EGF, laminin, TNF-α, ADAM10 • ADAM12: Collagen IV, DLL1, fibronectin, HB-EGF • ADAM15: Amphiregulin, CD23, collagen IV, E-cadherin, HB-EGF, ADAM10 • ADAM19: TNF-α, kit-ligand 1, TRANCE • ADAM28: IGBP3, VWF, CD23 • ADAM33: IL18	• Cell membrane (ADAM8, 12, 20, 21, 28, 30, 33) • Cytosol (ADAM8, 9, 15, 19) • Nucleus (ADAM33) • Endoplasmic reticulum (ADAM9) • Vesicles (ADAM9, 15, 19) • Mitochondria (ADAM28)	• Secreted to ECM (ADAM8) • Secreted in female reproductive system (ADAM12) • Secreted to blood (ADAM28)
	Type II	ADAM2, 7, 11, 18, 22, 23, 27, 29, 32	Inactive	• Cell membrane (ADAM2, 7, 11, 18, 22, 23, 29, 32) • Cytosol (ADAM18, 22, 23, 29, 32)	• Secreted to ECM (ADAM2, 22, 23)
	Type III	ADAM15	Amphiregulin, CD23, collagen IV, E-cadherin, HB-EGF, ADAM10	• Cell membrane • Cytosol • Vesicles	• Unknown
	Type IV	ADAM10, 17	• ADAM10: Amyloid precursor protein, betacellulin, CD23, CD30, CD44, DLL1, E-cadherin, EGF, Fas-L, HB-EGF, HER2, L1, N-cadherin, Notch, TNF-α • ADAM17: Amphiregulin, amyloid precursor protein, CD44, collagen XVII, DLL1, epiregulin, epigen, HB-EGF, ICAM-1, L-selectin, notch, TGF-α, TNF-α, VCAM1	• Cell membrane (ADAM10, 17) • Cytosol (ADAM10, 17) • Vesicles (ADAM10)	• Unknown

TABLE 3.1 Metalloproteases characterization in terms of substrate, subcellular location, and secretion. The information related to subcellular location and secretion was extracted from Human Protein Atlas (HPA) and cansar.ai.—cont'd

Metalloproteases type	Metalloproteases subtype	Protease	Main natural substrates	Subcellular location (HPA and cansar.ai)	Secretion (HPA and cansar.ai)
ADAMTS	Aggrecanases and proteoglycanases	ADAMTS1, 4, 5, 8, 9, 15, 20	Proteoglycans	• Cell membrane (ADAMTS1, 4) • Cytosol (ADAMTS1, 4, 9, 15, 20) • Nucleus (ADAMTS4) • Nucleoplasm (ADAMTS5) • Vesicles (ADAMTS9) • Endoplasmic reticulum (ADAMTS9)	• Secreted to ECM (ADAMTS1, 4, 5, 8, 9, 15, 20)
	COMP proteinases	ADAMTS7, 12	COMP	• Cytosol (ADAMTS7) • Vesicles (ADAMTS7) • Nucleoli (ADAMTS12) • Mitochondria (ADAMTS12)	• Secreted to ECM (ADAMTS7, 12)
	Procollagen N-peptidases	ADAMTS2, 13, 14	Procollagen	• Cell membrane (ADAMTS2) • Cytosol (ADAMTS2, 13, 14) • Vesicles (ADAMTS2, 13)	• Secreted to ECM (ADAMTS2, 14) • Secreted to blood (ADAMTS13)
	von Willebrand factor proteinase	ADAMTS13	VWF	• Cytosol • Vesicles	• Secreted to blood
	Other ADAMTS	ADAMTS6, 10, 16, 17, 18, 19	Not defined	• Cytosol (ADAMTS10, 17, 18) • Nucleoplasm (ADAMTS17) • Vesicles (ADAMTS16, 19) • Midbody, mitotic spindle (ADAMTS18, 19)	• Secreted to ECM (ADAMTS6, 10, 16, 17, 18, 19

ADAMs, disintegrin and metalloproteases; *ADAMTSs*, disintegrin and metalloproteinase with thrombospondin motifs; *CD23*, Fc epsilon RII; *CD30*, TNF receptor superfamily member 8; *COMP*, cartilage oligomeric matrix protein; *DLL1*, delta-like canonical notch ligand 1; *ECM*, extracellular matrix; *EGF*, epidermal growth factor; *HB-EFG*, heparin-binding EGF-like growth factor; *HER2*, receptor tyrosine-protein kinase erbB-2; *IGBP3*, insulin-like growth factor—binding protein 3; *IL18*, interleukin-18; *IL-1β*, interleukin-1 beta; *L1*, L1 cell adhesion molecule; *MMPs*, matrix metalloproteases; notch, neurogenic locus notch homolog protein 1; *TGF-α*, transforming growth factor alpha; *TNF-α*, tumor necrosis factor alpha; *TRANCE*, TNF-related activation-induced cytokine; *VCAM1*, vascular cell adhesion molecule 1; *VWF*, von Willebrand factor.

proMMP3. They are also responsible for cleaving collagen, gelatin, elastin, laminin, fibronectin, and fibrin (Nagase et al., 2006; Groblewska et al., 2012). Increased MMP9 expression levels are associated with a worse prognosis in non−small-cell lung cancer, cervical cancer, breast cancer, papillary thyroid carcinoma, gastric cancer, ovarian cancer, colorectal cancer, invasive pituitary adenoma, and glioma (Jackson et al., 2017; DI Carlo, 2012; Chang et al., 2020; Jiang and Li, 2021). In the case of MMP2, this proteinase is also associated with various types of cancer, namely breast cancer, cervical cancer, colorectal cancer, endometrial cancer, gastric cancer, gynecological cancer, glioma, invasive pituitary adenoma, non−small-cell lung cancer, ovarian cancer, and renal cell carcinoma (Jia et al., 2017; Fouad et al., 2019; Gao et al., 2012; Liu, 2016; Liu et al., 2018, 2023). MMP7 may be useful as a prognostic biomarker in colorectal cancer, esophageal cancer, gastric cancer, lung cancer, and urothelial cancer (Kubik et al., 2023; Miao et al., 2015; Soleyman-Jahi et al., 2015; Sun et al., 2015). There is also some literature on MMP1 and MMP14 (Chang et al., 2020; Dong et al., 2015; Hsiao et al., 2020; Wang et al., 2021). MMP1 is related to cervical cancer and colorectal cancer. While increased levels of MMP1 are associated with a worse prognosis in cervical cancer, a lack of MMP1 is associated with worse outcomes in colon cancer. Increased levels of MMP14 may indicate a worse prognosis in gastric cancer.

ADAMs are proteases belonging to the subfamily of the metzincins superfamily of metalloproteases involved mainly in the processes of proteolysis and cell adhesion. All ADAMs contain a disintegrin domain that interacts with elements of the extracellular matrix and integrins. Adhesion takes place via the link between the disintegrin domain and integrins. While MMPs are responsible for cleaving extracellular matrix proteins, ADAMs act on ectodomains of type I and type II transmembrane proteins. ADAMs, especially ADAM10 and ADAM17, have been studied for their potential as cancer biomarkers (Duffy et al., 2011; Mullooly et al., 2016; Zhong and Khalil, 2019). ADAM17 overexpression is predictive of poor prognosis in gastric cancer (Ni et al., 2020). Increased levels of BUB1 mitotic checkpoint serine/threonine kinase B (BUB1B) are associated with worse overall survival and progression-free survival in lung cancer and can be used as a prognostic biomarker.

ADAMTs are secreted proteases that also regulate the composition of the extracellular matrix. There are 19 genes that encode ADAMTS, and these are grouped into six different families. All ADAMTs are made up of a prodomain, disintegrin, thrombospondin repeats (TSR), a cysteine-rich domain, and a spacer. Type I is made up of these elements. The other types, in addition to these elements, have GON-1 domains (type II); procollagen N-propeptidase (type III); complement C1r/C1s, Uegf, Bmp1 (CUB) domains (type IV); mucin/proteoglycan domain; and protease and lacunin (type V). The difference between type VI and type V is that it only has the PLAC domain. In terms of structure, ADAMTs are like ADAMs, but instead of having an EGF-like region and a highly variable region (HVR), they have TSR and a spacer (Rakash, 2012).

3. Serine proteases

Serine proteases are the second largest family of proteases. Serine proteases play a very important role in cancer. The main serine proteases are trypsin, trypsin-like, granzyme B (GZMB), elastase (ELANE), cathepsin G (CSTG), kallikreins (KLKs), prostate-specific antigen

(PSA), urokinase-type plasminogen activator (uPA), fibroblast activation protein alpha (FAP), rhomboid proteases, high-temperature requirement A (HtrA), matriptases, and hepsins, as described in Table 3.2. They are involved in various processes, including ECM remodeling, signaling pathways, proteolytic cascade, and immunosuppressive effects. Their activity is regulated by their inhibitors, serine protease inhibitors (SERPINS). They can be divided into three groups: type I (carboxy-terminal transmembrane domain), type II (amino-terminal proximal signal anchor), and GPA-anchored (Martin and List, 2019; Tagirasa and Yoo, 2022; Vizovisek et al., 2021). KLKs have been extensively studied in ovarian cancer and prostate cancer. PSA is one of the most studied biomarkers in prostate cancer, and it is encoded by the KLK3 gene. This proteinase is produced by the prostate gland, and its function is to break down seminal proteins to promote the release of sperm. Increased PSA levels have been associated with inflammatory processes, benign prostatic hyperplasia, and prostate cancer. The use of PSA as a prostate cancer screening is somewhat controversial due to the overdiagnosis of prostate cancer, with some patients being treated unnecessarily because they have insignificant tumors (Fenton et al., 2018; Kadalayil et al., 2017). There are several guidelines that recommend the PSA test for the detection of early-stage prostate cancers. However, the guidelines are not consistent in terms of the age range that should be used as a criterion for screening. Depending on the country, there is a wide variation in how this screening is applied (Tikkinen et al., 2018). In the case

TABLE 3.2 Serine proteases characterization in terms of substrate, subcellular location, and secretion. The information related to subcellular location and secretion was extracted from Human Protein Atlas (HPA) and cansar.ai.

Serine protease type	Protease	Main natural substrates	Subcellular location (HPA and cansar.ai)	Secretion (HPA and cansar.ai)
Trypsin, trypsin-like	PRSS1, PRSS2, PRSS3	Digestion of proteins in small intestine	• Cytosol (PRSS1, 2, 3) • Endoplasmic reticulum (PRSS1, 2, 3) • Vesicles (PRSS1, 2, 3)	• Secreted to digestive system (PRSS1, 2, 3)
Granzyme B	GZMB	PARP, DNA-PK, nucleophosmin, topoisomerase 1, nucleolin, ICP4, NUMA, DBP, fibronectin, laminin, vitronectin, aggrecan, PAR1, VWF, plasminogen	• Cytosol • Cell membrane • Nucleus • Other organelles	• Secreted to blood
Elastase, neutrophil expressed	ELANE	ECM proteins (elastin, basement membrane proteins), proteoglycans, azurophil granules, OmpA of *Escherichia coli*, virulence factors	• Cytosol	• Secreted to blood
Cathepsin G	CSTG		• Cytosol • Cell membrane • Nucleus • Other organelles	• Secreted
Cathepsin A	CSTA	Endothelin-I	• Cytosol • Vesicles	• Secreted to blood

(*Continued*)

TABLE 3.2 Serine proteases characterization in terms of substrate, subcellular location, and secretion. The information related to subcellular location and secretion was extracted from Human Protein Atlas (HPA) and cansar.ai.—cont'd

Serine protease type	Protease	Main natural substrates	Subcellular location (HPA and cansar.ai)	Secretion (HPA and cansar.ai)
KLK	KLKB1, KLK 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15	HK, scuPA, factor XI, IGF-BP, IGF-1	• Cell membrane (KLK6, 7, 10) • Nucleus (KLK1, 3, 6) • Cytosol (KLKB1, KLK2, 4, 5, 7, 8, 10, 11, 13, 15) • Nucleoplasm (KLK6) • Nuclear membrane (KLK6, 7) • Cytokinetic bridge (KLK6) • Vesicles (KLK8) • Other organelles (KLK11)	• Secreted to digestive system (KLK1) • Secreted in male reproductive system (KLK2, 3, 4) • Locally secreted (KLK5, 6, 7, 8, 9, 10, 11, 12, 13, 14) • Secreted to blood (KLKB1, KLK15)
uPA	PLAU	Plasminogen	• Cytosol • Cell membrane	• Secreted to blood
tPA	PLAT		• Cytosol • Actin filaments	• Secreted to blood
Plasmin	PLG	β-casein, α_{s2}-casein	• Cytosol • Vesicles	• Secreted to blood
Dipeptidyl peptidases	DPP3, 4, 6, 7, 8, 9, 10	Chemokines, neuropeptides, peptide hormones	• Cytosol (DPP3, 4, 6, 7, 8, 9, 10) • Cell membrane (DPP3, 6, 10) • Nucleus (DPP3, 10) • other organelles (DPP6, 7)	• Secreted to blood (DPP4)
Fibroblast activation protein alpha	FAP	Denatured type I collagen, α2-antiplasmin, FGF-21	• Cytosol • Cell membrane	• Secreted to blood
Rhomboid protease	RHBDL1, RHBDL2, RHBDL3, RHBDL4	APP, subunits of the oligosaccharyltransferase complex, pTCRa, TCRa, opsin-degron, MPZ-L170R, TSAP6, proTGFa, BIK1, thrombomodulin, ephrin B2/B3, EGFR, EGF, CLEC14A	• Golgi (RHBDL1, 3) • Plasma membrane (RHBDL2, 3) • Endosomes (RHBDL3) • Endoplasmic reticulum (RHBDL2, 4)	Unknown

TABLE 3.2 Serine proteases characterization in terms of substrate, subcellular location, and secretion. The information related to subcellular location and secretion was extracted from Human Protein Atlas (HPA) and cansar.ai.—cont'd

Serine protease type	Protease	Main natural substrates	Subcellular location (HPA and cansar.ai)	Secretion (HPA and cansar.ai)
HtrA	HTRA1, HTRA2, HTRA3, HTRA4	Fibronectin, type I collagen, decorin, biglycan, EFEMP1 and TSP1, LTBP-1, APP, tau protein aggregates, XIAP, IAPs, HAX-1, Ped-Pea15, WTP1, actin, β-tubulin, vimentin, BMP4, TGF-β1, TGF-β2, GDF5, VE-cadherin, VEGF-A receptor KDR	• Cell membrane (HTRA1, 2) • Cytosol (HTRA1, 3) • Mitochondria (HTRA2) • Vesicles (HTRA3)	• Secreted to blood (HTRA1) • Locally secreted (HTRA3) • Secreted in female reproductive system (HTRA4)
Matriptases	ST14, TMPRSS6	Protease-activated receptor-2, pro-urokinase plasminogen activator, pro-hepatocyte growth factor, pro-prostasin, pro-filaggrin, transmembrane and associated with Src kinases (Trask/CD318/SIMA135/CDCP-1), proMSP-1	• Cytosol (ST14) • Nucleoplasm and nucleus (ST14) • Vesicles (ST14) • Cell membrane (ST14, TMPRSS6)	• Secreted (TMPRSS6)
Hepsins	HPN	Blood coagulation factors VII, IX and XII, pro-urokinase, pro-hepatocyte growth factor (pro-HGF), prostasin, epidermal growth factor receptor, laminin, liver microsomal glutathione transferase	• Cell membrane • Other organelles membrane • Cytosol	• Secreted
Proteinase 3	PRTN3	Plasma proteins, cytokines and heir receptors, TIMPs, CD2, CD4, CD8, complement receptors, macrophage phosphatidyl serine receptor, protease activated receptors	• Cell membrane • Cytosol • Vesicles	• Secreted to blood
Tryptase	TPSAB1, TPSB2, TPSG1, TPSD1	VIP, calcitonin gene-related peptide, fibronectin, kininogens, PAR2	• Cell membrane (TPSG1) • Cytosol (TPSD1) • Nucleus (TPSD1) • Vesicles (TPSAB1)	• Secreted to blood (TPSAB1, TPSB2) • Secreted (TPSG1)
Chymase	CMA1	Type VI collagen, aggrecan, proMMP1,- 3, and -9	• Cytosol	• Secreted to blood
Thrombin	F2	Coagulation factors V, VIII, XI and XIII, protein C, fibrinogen, protease-activated receptors PAR1, -3, and -4	• Cytosol • Other organelles	• Secreted to blood

(*Continued*)

TABLE 3.2 Serine proteases characterization in terms of substrate, subcellular location, and secretion. The information related to subcellular location and secretion was extracted from Human Protein Atlas (HPA) and cansar.ai.—cont'd

Serine protease type	Protease	Main natural substrates	Subcellular location (HPA and cansar.ai)	Secretion (HPA and cansar.ai)
Complement serine proteases	C1r, C1s, MASPs 1–3, C2, CFB, CFD, CFI	Clr, C1, C2, C3, C3b, C4, C4b, C5, MASP1, MASP2	• Cell membrane (CFD, CFI) • Cytosol (C1r, C1s, MASP1, C2, CFD) • Nucleoplasm (C1r) • Nucleus (C1s, MASP1) • Golgi apparatus (MASP2) • Endoplasmic reticulum (CFB) • Vesicles (CFB) • Cell junctions (CFB)	• Secreted (C1r) • Secreted to blood (C1s, MASP1, MASP2, C2, CFB, CFD, CFI)

APP, amyloid beta precursor protein; *BIK1*, botrytis-induced kinase 1; *C1*, complement component 1; *C2*, complement component 2; *C3*, complement component 3; *C4*, complement component 4; *C5*, complement component 5; *CD2*, cluster of differentiation 2; *CD4*, clusters of differentiation 4; *CD8*, clusters of differentiation 8; *CLEC14A*, C-type lectin/C-type lectin-like domain; *DBP*, D-Box binding PAR BZIP transcription factor; *DNA-PK*, DNA-dependent protein kinase; *ECM*, extracellular matrix; *EGF*, epidermal growth factor; *EGFR*, epidermal growth factor receptor; *FGF-21*, fibroblast growth factor; *ICP4*, infected cell protein 4; *IGF-1*, insulin-like growth factor 1; *IGF-BP*, insulin-like growth factor binding protein; *MASP1*, mannose-associated serine protease 1; *MASP2*, mannose-associated serine protease 2; *MPZ-L170R*, APP wild-type, chimeric mutant; *NUMA*, nuclear mitotic apparatus protein; *OmpA*, outer membrane protein A; *PAR*, protease activated receptor; *PARP*, poly [ADP-ribose] polymerase 1; *pTCRa*, pre–T cell antigen receptor alpha; *scuPA*, single-chain urokinase-type plasminogen activator; *TCRa*, T cell antigen receptor alpha; *TIMPs*, tissue inhibitors of metalloproteinases; *VIP*, vasoactive intestinal peptide; *VWF*, von Willebrand factor.

of prostate cancer, kallikreins may be useful as a diagnostic biomarker and in the case of ovarian cancer as a biomarker of ovarian cancer (Wu et al., 2015). Granzyme B is a serine protease commonly found in natural killer (NK) and cytotoxic cells. It is involved in cell death processes mediated by cytotoxic lymphocytes. In the case of head and neck cancer, overexpression of GZMB is associated with a better prognosis (Bisheshar et al., 2020). Some studies have been performed to evaluate the correlation between the tumor microenvironment and prognosis. $GZMB^+$ tumor-infiltrating lymphocytes are also associated with a better prognosis in follicular lymphoma and hepatocellular carcinoma (Ding et al., 2018; Xie et al., 2020) The uPA system is associated with the development and progression of cancer. This system is made up of uPA and the urokinase receptor (uPAR). uPAR is found on the surface of cells. When uPAR binds to uPA, it promotes its activation, which triggers the transformation of plasminogen into plasmin. Plasmin in turn is involved in ECM degradation and MMP activation. There are two endogenous inhibitors that specifically inhibit uPA, namely plasminogen activator inhibitor 1 (PAI-1) and plasminogen activator inhibitor 2 (PAI-2). High levels of uPA are associated with a worse prognosis in lymph node–negative breast cancer, gastroesophageal cancer, and non–small-cell lung cancer (Brungs et al., 2017; Look et al., 2002; Lu et al., 2017). Thrombin is a Na^+-activated serine protease that is involved in various cellular processes such as cell proliferation, angiogenesis, and metastasis. Its action is carried out through protease-activated receptors (PARs), which

are activated when serine proteases are in low concentration. Thrombin activates PAR1, PAR3, and PAR4. PAR2 is activated by trypsin. PAR1 and PAR2 are present in many types of cancer. Overexpression of PAR1 seems to be associated with a worse prognosis in ovarian, breast, melanoma, and prostate cancer. These two proteases are expressed in the same types of tumors. As for PAR3, its expression is more pronounced in kidney and liver cancer. Regarding PAR4, more studies are needed (Elste and Petersen, 2010). SPARC-like protein 1 (SPARCL1) is associated with the prognosis of gastrointestinal malignancies, its expression being predictive of a better prognosis (Hu et al., 2018). In prostate cancer, osteopontin (OPN) was associated with a higher Gleason score, higher TNM stage, higher Whitmore-Jewett stage, higher lymph node metastasis cases, and distant metastasis (Yu et al., 2021). Liver kinase B1 (LKB1), HtrA1 and transmembrane protease, and serine 4 (TMPRSS4) are associated with a wide variety of solid tumors (Altobelli et al., 2018; Zeng et al., 2016; Ren et al., 2019).

4. Cysteine proteases

The cysteine proteases most associated with carcinogenesis are caspases, cathepsins, and calpains. The main cysteine proteases are characterized in Table 3.3. Caspases (CASPs) can be initiator (CASP-2, -8, -9, and -10) or effector (CASP-3, -6 and 7). When there is a signal, the former are activated while the effectors carry out the apoptotic response that culminates in cell death. Each caspase comprises a prodomain and a catalytic domain. CASP-3 is related to head and neck cancer and renal cell carcinoma. CASP-3 favors the malignancy of oral premalignant lesions for head and neck cancer. However, it is statistically significantly related to survival outcomes (Silva et al., 2022). Cathepsins are involved in the processes of cell progression, proliferation, and invasion. This group of cysteine proteases consists of cathepsin B, C, F, H, K, L, O, S, V, X, and W. Cathepsin levels are increased in tumors, and this overexpression is associated with a worse prognosis in head and neck, breast, lung, and colorectal cancer (Olson and Joyce, 2015; Rudzińska et al., 2019; Tan et al., 2013). The relationship between cathepsin D and prognosis in breast cancer is well established (Ferrandina et al., 1997; Masson et al., 2011). Calpains are a group of intracellular proteases that are activated by calcium. Calpain-1 and -2 are the main types of calpains. Overexpression of calpains in the tumor is associated with a worse prognosis, namely, shorter survival. Like cathepsins, calpains are involved in the processes of carcinogenesis and metastasization. They are activated by an increase in Ca^{2+} concentration, leading to the activation of pathways such as phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) that are associated with cell death. On the other hand, they also regulate the activity of some cytokines and are also involved in the immune response (Leloup and Wells, 2011; Shapovalov et al., 2022; Tagirasa and Yoo, 2022). Overexpression of calpain small subunit 1 (CAPN4) is associated with a worse prognosis, particularly in gastrointestinal cancer (Tang et al., 2019).

5. Aspartic proteases

The main aspartic proteases originate in the kidney (renin, napsin A), lysosomes (cathepsins D [CTSD] and E [CSTE]), stomach (pepsin, gastricsin, and chymosin) and lung (napsin A

TABLE 3.3 Cysteine proteases characterization in terms of substrate, subcellular location, and secretion. The information related to subcellular location and secretion was extracted from Human Protein Atlas (HPA), cansar.ai and ProteomicsDB.

Cysteine protease type	Protease	Main natural substrates	Subcellular location (HPA and cansar.ai)	Secretion (HPA, cansar.ai, ProteomicsDB)
Caspases	CASP1, 4, 5	(W/L)EXD motifs	• Cell membrane (CASP4) • Cytosol (CASP1, 4, 5) • Nucleoplasm (CASP1)	• Secreted to blood (CASP1, 4, 5)
	CASP6, 8, 9, 10	(I/V/L)EXD motifs	• Cytosol (CASP6, 8, 9, 10) • Nucleoplasm (CASP6, 8) • Nucleus (CASP6) • Mitochondria (CASP9) • Golgi apparatus (CASP10) • Vesicles (CASP10)	• Secreted to blood (CASP6, 8, 9, 10)
	CASP3, 7	DEXD motifs	• Cytosol (CASP3, 7) • Nucleoplasm (CASP3) • Nucleus (CASP7) • Mitochondria (CASP3)	• Secreted to blood (CASP3, 7)
	CASP2	VDVAD motifs	• Cytosol • Nucleus • Mitochondria	• Secreted to blood
	CASP14	WEHD motifs	• Cytosol • Nucleoplasm • Mitochondria	• Secreted to blood
Cathepsins	CTSB	Trypsinogen, hemoglobin, azo-casein, azo-hemoglobin, cartilage proteoglycan, collagen, gelatin, immunoglobulin G, myosin, bacterial cell wall protein	• Cytosol • Nucleus • Nucleoli • Vesicles	• Secreted to blood
	CTSC	Neutrophil serine proteases such as cathepsin G, proteinase 3, and elastase	• Cell membrane • Cytosol • Vesicles	• Secreted to blood
	CTSF	LAMP2, lip10	• Cell membrane • Cytosol • Endoplasmic reticulum	
	CTSH	Surfactant protein B	• Cytosol • Cytoplasmic bodies • Vesicles	• Secreted to blood
	CTSK	Kinins, elastin, collagen, gelatin	• Cell membrane • Cytosol • Vesicles	• Secreted to blood
	CTSL	Wide range of enzymes, receptors, and transcription factors	• Cell membrane • Cytosol • Nucleus • Golgi apparatus • Vesicles	• Secreted to blood

TABLE 3.3 Cysteine proteases characterization in terms of substrate, subcellular location, and secretion. The information related to subcellular location and secretion was extracted from Human Protein Atlas (HPA), cansar.ai and ProteomicsDB.—cont'd

Cysteine protease type	Protease	Main natural substrates	Subcellular location (HPA and cansar.ai)	Secretion (HPA, cansar.ai, ProteomicsDB)
	CTSS	Albumin, collagen type I, CX3CL1, elastin, hemoglobin, li protein, insulin, laminin, myelin	• Cytosol • Vesicles	• Secreted to blood
	CTSV	Elastin, gelatin, neuropeptide Y, plasminogen, proenkephalin	• Cell membrane • Cytosol • Nucleoplasm • Nucleoli fibrillar center	• Unknown
	CTSX	Regulatory motifs at C-terminus, β2 integrin receptor Mac-1 (CD11b/CD18), β2 integrin receptor LFA-1 (CD11a/CD18)	• Cell membrane • Vesicles	• Secreted
	CTSW	Unknown	• Cell membrane • Cytosol	• Secreted to blood
Calpains	CAPN1, CAPN2	Cytoskeletal and associated proteins, kinases and phosphatases, membrane receptors and transporters, and steroid receptors	• Cytosol (CAPN1, 2)	• Secreted to blood (CAPN1, 2)

CX3CL1, C-X3-C motif chemokine ligand 1; *LAMP2*, X-linked lysosome-associated membrane protein-2; *lip10*, fragment of the MHC class II-associated invariant chain.

[NAPSA]). These aspartic proteases are described in Table 3.4. CSTD is an endoprotease present in lysosomes, which is responsible for the degradation of proteins in an acidic environment. This proteinase is involved in the processes of cell proliferation, invasion, and metastasis and angiogenesis (Benes et al., 2008). High levels of CSTD are associated with worse prognosis in breast, head and neck, lung, and colorectal cancer. A metaanalysis validated CSTD as a prognostic biomarker for breast cancer. CSTD overexpression was associated with worse overall survival (OS) and disease-free survival (DFS) (Ferrandina et al., 1997; Kang et al., 2020). The link with breast cancer is partly because CSTD is extensively excreted by human epithelial breast cancer cells (Masson et al., 2011; Radisky and Radisky, 2010). On the other hand, overexpression of CSTE has been associated with pancreatic adenocarcinoma and has the potential to be used as an early diagnosis biomarker in pancreatic adenocarcinoma (Cruz-Monserrate et al., 2012). NAPSA has been widely studied in cancer, particularly endometrial, lung, and kidney cancer. In the case of endometrial cancer, it has shown potential as a biomarker for endometrial clear cell carcinoma. NAPSA can be used to distinguish between endometrial cancer and endometrioid and serous carcinoma (Travaglino et al., 2022). In clear cell ovarian cancer, NAPSA together with U3 small nucleolar ribonucleoprotein protein (IMP3) and hepatocyte nuclear factor 1 beta proved to be a highly specific panel for diagnosing this type of cancer (Butler et al., 2022). In lung cancer, a meta-analysis performed by Li et al. showed that NAPSA together with thyroid transcription factor

TABLE 3.4 Aspartic proteases characterization in terms of substrate, subcellular location, and secretion. The information related to subcellular location and secretion was extracted from Human Protein Atlas (HPA), cansar.ai and Proteomics DB.

Aspartic protease	Protease	Main natural substrates	Subcellular location (HPA and cansar.ai)	Secretion (HPA and cansar.ai)
Renin	REN	Angiotensinogen	• Cytosol	• Secreted to blood
Napsin A	NAPSA	N-terminal propeptide of proSP-B	• Organelles	• Secreted to blood
Cathepsin D	CTSD	α-Synuclein, amyloid precursor protein, angiostatin, apolipoprotein E, CASP-8, prolactin, tau protein	• Cytosol • Organelles	• Secreted to blood
Cathepsin E	CTSE	Wide range of proteins	• Cytosol • Vesicles	• Secreted to blood
Pepsin	PGA5	Wide range of proteins	• Cytosol	• Secreted to digestive system
Gastricsin	PGC	Wide range of proteins	• Cytosol • Nucleus	• Secreted to digestive system

1 (TTF-1) can be used to distinguish lung adenocarcinoma from squamous cell carcinoma (Li et al., 2014a,b,c).

6. Threonine proteases

Proteasomes are the most important threonine proteases in the context of cancer. The proteasome is a multisubunit enzyme complex whose main function is to degrade ubiquitinated proteins that are recognized by the ubiquitin-conjugation system (UPS). The 26S proteasome is made up of the 20S catalytic component and the 19S regulatory component. The UPS activates the free ubiquitin protein and transfers it to the target protein via the ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2) and ubiquitin-protein ligase (E3). E1 binds to ubiquitin, which activates it and transfers it to E2, which in turn transfers it to the target substrate. Polyubiquitinated proteins are recognized by proteasome 20S that removes the ubiquitin and degrades the target protein as shown in Fig. 3.2. Proteosomes participate in the regulation of various biological processes associated with cancer, such as the cell cycle and apoptosis. There are some drugs that participate in the inhibition of the proteosome, more particularly in the catalytic activity of the 20S subunit. The best known are proteasome inhibitors such as MG-132 ((S,R,S)-(-)-MG-132, Z-Leu-D-Leu-Leu-al) and PSI (N-carbo-benzoxy-L-gamma-t-butyl-L-glutamyl-L-alanyl-L-leucinal), lactacystin, TMC-95A, eponemycin, NLVS (4-hydroxy-5-iodo-3-nitrophenylacetyl-Leu-Leu-leucinal-vinyl sulfone), epoxomicin, and peptide boronic acids (Adams, 2003; Mani and Gelmann, 2005).

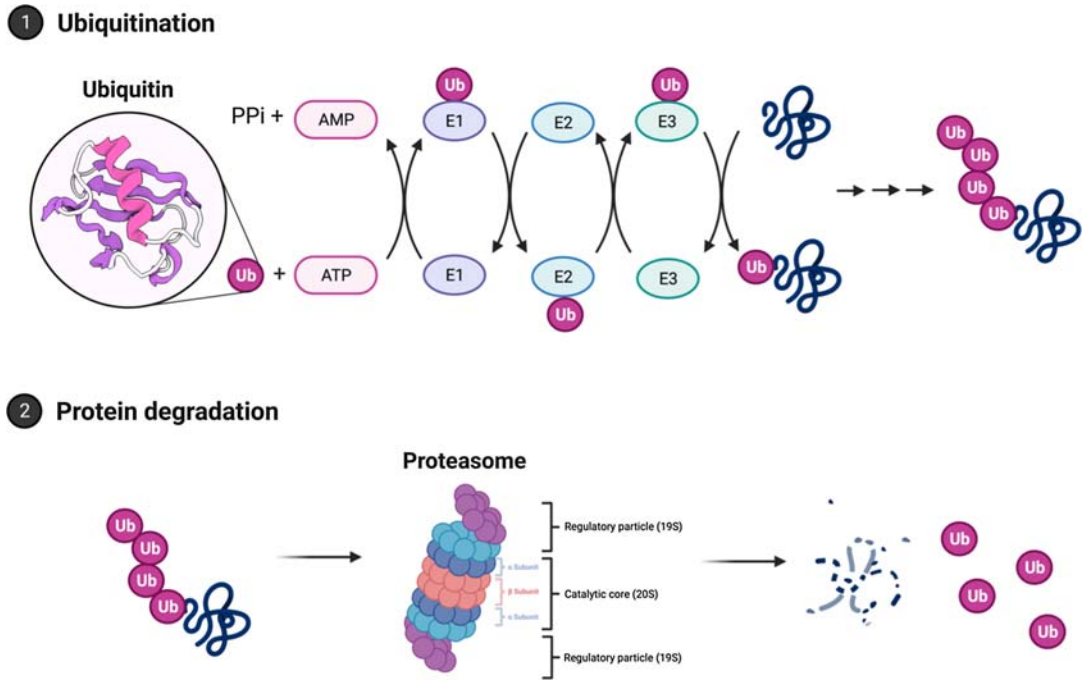

FIGURE 3.2 Ubiquitination and protein degradation process. (1) Ubiquitination consists of binding ubiquitin to the E1 enzyme, which is then transferred to the E2 enzyme and finally to the E3 enzyme, which transfers the ubiquitin to the target protein. (2) The protein of interest bound to ubiquitin is degraded in the proteosome and the ubiquitin recycled. *Created in BioRender.*

7. Biomarker value of for different types of cancer

The proteases with high-evidence literature pointing the potential of their use as biomarkers are summarized in Brungs et al. (2017), Chen et al. (2015), (2021a,b,c), Dong et al. (2015), Eisenach et al. (2013), Fouad et al. (2019), Fu et al. (2015), Gong et al. (2016), Hu et al. (2017), Jia et al. (2017), Jiang and Li (2021), Kubik et al. (2023), Lee et al. (2013), Li et al. (2013a,b), (2014a,b,c), Liang and Chang (2018), Liang et al. (2014), Liu (2016), Liu et al. (2016a,b), (2017), (2018), (2023), Long et al. (2014), Lu et al. (2015), (2017), Miao et al. (2015), Ni et al. (2020), Peng et al. (2012), (2013), Qian et al. (2010), Ran et al. (2019), Ren et al. (2015), (2019), Roddam et al. (2005), Russo et al. (2017), Shen et al. (2014), Shi et al. (2013), Shin et al. (2021), Silva et al. (2022), Soleyman-Jahi et al. (2015), Stephan et al. (2005), Sun et al. (2015), Wang et al. (2014a,b), (2020), (2021), Wen et al. (2023), Wong et al. (2011), Yang et al. (2015), Yi et al. (2017), Yu et al. (2021), Zeng et al. (2016), Zhang et al. (2012a,b,c), Zheng et al. (2015), Zhou et al. (2018) (*Prostate specific antigen as a biomarker for breast cancer: a meta-analysis study*, no date). Universal stress proteins (USPs), such as USP7 and USP39, and C-Jun activation domain-binding protein-1 (Jab1) have associated a several types of tumors (Kisaï and Koji, 2021; Remitha et al., 2023; Yu et al., 2021). In fact, most of the proteases lack cancer specificity with few exceptions. The proteases shown

in Fig. 3.3 correspond to the proteases that show the greatest correlation with the processes related to cancer development, progression, and metastasization. PSA is the most well-known protease due to its widespread use as a screening method for prostate cancer. Although it is a highly sensitive biomarker, it is not very specific (Merriel et al., 2022). The use of biomarker panels may be capable to overcome the lack of specificity observed when proteases are used as isolated biomarkers.

Besides looking to proteases overexpression, some studies focus on the analysis of proteases gene variants. In fact, there are many variants of genes that code for proteases under investigation to assess their relationship with the various types of cancer that exist. The most studied polymorphisms are for caspases and metalloproteases. Regarding caspases, CASP-8 is the one with the most scientific evidence. The CASP-8-652 6N ins/del polymorphism is associated with a lower risk of cancer, especially renal cell carcinoma and breast cancer (De Martino et al., 2013). The CASP-8 D302H polymorphism is also associated with a lower risk of breast cancer. However, it is associated with susceptibility to prostate cancer. In the Asian population, the rs7608692*A polymorphism may be a risk factor for developing cancer (Camp et al., 2012; Sergentanis and Economopoulos, 2010). Conversely, the rs3834129*, rs6704688*T, rs3769827*C, and rs3769825*C polymorphisms may be cancer-protective factors. The CASP-9 rs4645981 and rs1052571 polymorphisms are associated

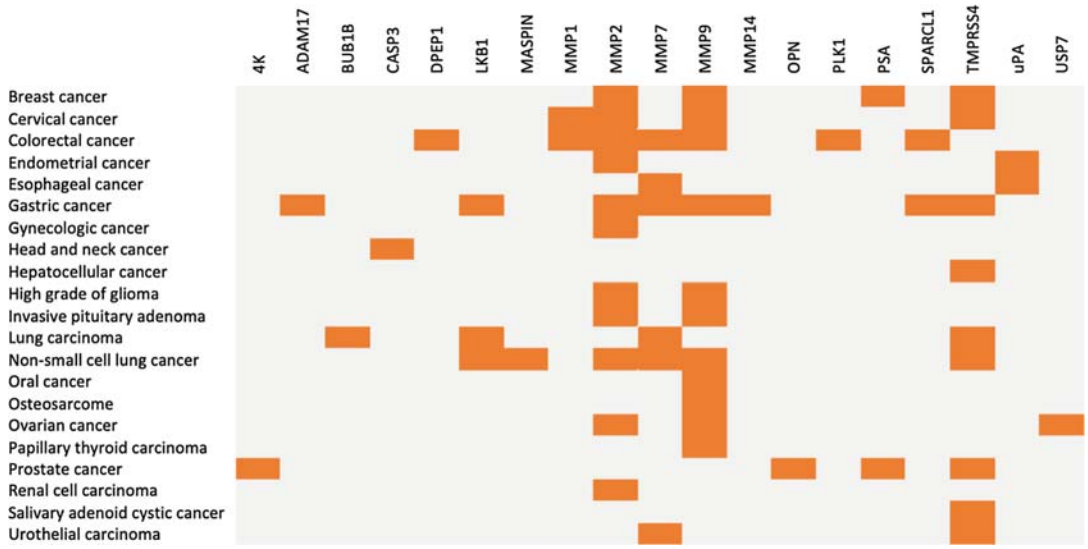

FIGURE 3.3 Proteases with high-evidence literature confirming the potential to be used as biomarkers in cancer. In *orange* are biomarkers with high-evidence literature supporting their use as prognostic and/or diagnostic biomarkers in a particular type of cancer. In *gray* are biomarkers with no clear association with tumors shown in the *image*. *4K*, four-kallikrein; *ADAM17*, ADAM metallopeptidase domain 17; *BUB1B*, mitotic checkpoint serine/threonine-protein kinase BUB1 beta; *CASP3*, caspase-3; *DPEP1*, dipeptidase 1; *LKB1*, liver kinase B1; *MASPIN*, mammary serine protease inhibitor; *MMP1*, matrix metallopeptidase 1; *MMP14*, matrix metallopeptidase 14; *MMP2*, matrix metallopeptidase 2; *MMP7*, matrix metallopeptidase 7; *MMP9*, matrix metallopeptidase 9; *OPN*, osteopontin; *PLK1*, polo-like kinase 1; *PSA*, prostate-specific antigen; *SPARCL1*, SPARC-like protein 1; *TMPRSS4*, transmembrane serine protease 4; *uPA*, urokinase-type plasminogen activator; *USP7*, ubiquitin-specific peptidase 7.

with greater susceptibility to developing cancer, while the CASP-9 rs4645978 polymorphism is a protective factor (Sargazi et al., 2021). The CASP-10 rs13006529*T polymorphism may be a risk factor for cancer, particularly breast cancer. CASP-3 rs2705897 polymorphism is associated with susceptibility to cancer, while rs1049216 seems to be the opposite (Yan et al., 2013). CASP-5, CASP-7, CASP-8, and CASP-9 are associated with lung cancer risk (Sargazi et al., 2021; Xu et al., 2012; Yan et al., 2012, 2013; Yin et al., 2010; Zhang et al., 2013a,b,c). The most studied metalloproteinases are MMP1, MMP2, MMP7, and MMP9. The MMP1-1607 1G > 2G polymorphism is associated with cancer risk, particularly colorectal cancer and head and neck cancer (Zhang et al., 2013a,b,c). The MMP1 rs1799705 polymorphism is associated with esophageal cancer risk (Lu et al., 2015). Regarding MMP2, the MMP2-1306 C > T polymorphism is associated with a lower risk of developing many cancers. However, it is associated with greater susceptibility to lung cancer, bladder cancer, and head and neck cancer. Regarding breast cancer, its role is not yet well established, since the existing studies are somewhat contradictory. The MMP2 2735 C > T polymorphism is also associated with lung cancer risk. Regarding MMP7, the MMP7-181 A > G polymorphism is the most studied. It is associated with an overall cancer risk, particularly for digestive system cancer and cervical cancer (Das et al., 2022; Ke et al., 2013; Yang et al., 2013). Of MMP9, the MMP9-1562 C/T polymorphism has been associated with greater susceptibility to digestive and breast cancer. On the other hand, it seems to be a protective factor for colorectal and lung cancer. MMP9 rs3918242 is also associated with an increased risk of breast cancer, while MMP9 rs3787268 appears to be a protective factor. The MMP8 rs11225395 polymorphism is associated with a higher risk of cancer (Feng et al., 2019a,b). In relation to angiotensin-converting enzyme (ACE), ACE I/D polymorphism is associated with susceptibility to cancer. However, some studies show that it is not associated with a risk of digestive system and lung cancer (Chen et al., 2021a,b,c; Ruiter et al., 2011; Xiao et al., 2019). There are other proteases that have also been studied, but with less emphasis. Genetic alterations in KLK15 are associated with prostate cancer (Batra et al., 2011). There is a strong correlation between ER-stress variants in the carboxypeptidase A1 (CPA1) and carboxypeptidase B1 (CPB1) genes and an increased risk of developing pancreatic cancer (Kawamoto et al., 2022). The ADAMTSL1 rs715212 polymorphism is associated with a worse prognosis in young women with recently treated breast cancer (Kadalayil et al., 2017). The study of polymorphisms in genes that encode proteases is very important for classifying these variants to identify pathogenic and potentially pathogenic variants.

8. Biological fluids as source of proteases biomarkers

8.1 Serum

Studies using serum as liquid biopsy predominantly evaluate metalloproteases. Serum MMP9 is the metalloprotease with most literature evidence supporting its use as cancer biomarker. Serum MMP9 has been associated with non−small-cell lung cancer, ovarian cancer, breast cancer, and colorectal cancer. In the case of ovarian cancer and breast cancer, it is useful in stratifying the prognosis of these patients. In the case of non−small-cell lung cancer, higher levels of MMP9 in the tissue are related to prognosis, but the same is not true for

MMP9 in serum. In colorectal cancer, it can be used as a diagnostic biomarker; however, it has only average diagnostic value. Serum MMP7 may be useful as a prognostic biomarker in advanced bladder cancer (El Demery et al., 2014). Serum PSA also seems to be associated with cancer. As a biomarker, it can be useful in the diagnosis of this type of cancer, as well as in the differential diagnosis with benign breast pathology (Ghallab and Shaker, 2017; Gong et al., 2016).

8.2 Saliva

Saliva has attracted increasing attention as liquid biopsy because it is easy to collect, is noninvasive, and allows multiple collections. Some proteases have been studied in saliva samples from cancer patients to understand their potential as diagnostic and prognostic salivary biomarkers. Most of the existing literature in this area concerns head and neck cancer. MMP1 and MMP3 levels are increased in oral cancer patients and have a high discriminatory power, allowing oral cancer patients to be identified from healthy individuals. MMP1 and MMP3 are diagnostic biomarkers for oral cancer, both when tumor tissue and saliva from oral cancer patients are analyzed. In the case of MMP1, this is also true when using dried saliva stains (DSS) (AlAli et al., 2020; Peisker et al., 2017; Shin et al., 2021; Smriti et al., 2020; Vuotila et al., 2002). The use of DSS has the advantage of controlling the degradation of salivary content by proteases, allowing the original content of the samples to be better preserved. It also has advantages in terms of sample preservation and storage, which is much easier than with traditional saliva collection methods. The detection of MMP1 in DSS has the potential to be used as a molecular diagnostic test for oral cancer (Hsiao et al., 2020). MMP9 has the potential to be used as a biomarker for diagnosis, prognosis, response to treatment in oral cancer patients and follow-up. MMP9 levels increase progressively among controls, patients with potentially malignant oral lesions and patients with oral cancer (AlAli et al., 2020; Ghallab and Shaker, 2017; Peisker et al., 2017; Shin et al., 2021; Smriti et al., 2020). MMP12 levels also show a similar pattern and can also be used as a diagnostic biomarker for oral cancer (Saleem et al., 2021). The levels of MMP8, MMP10, MMP13, and TIMP2 are increased in both the tissue and saliva of oral cancer patients, and there is a correlation between these levels and survival (Cai et al., 2022). These biomarkers could therefore be diagnostic and/or prognostic biomarkers for oral cancer. Cathepsin V (CTSV), KLK5, serine 11B transmembrane protease (TMPRSS11B), and ADAM9 could also be diagnostic biomarkers for oral cancer. TMPRSS11B has high levels in saliva samples from patients with glioblastoma, showing that it may be related to the prognosis of these patients (Müller Bark et al., 2023).

8.3 Urine

Over the past two decades, there has been an increase in the research focused on proteases using urine as a liquid biopsy. The urinary gene fusion transcripts involving v-ets erythroblastosis virus E26 oncogene homolog, commonly known as ERG, and the androgen-regulated gene transmembrane protease, serine 2 (TMPRSS2), have been studied as a prostate cancer biomarker. Urinary TMPRSS2:ERG has the potential to be used as a diagnostic biomarker for prostate cancer. Because it is related to tumor aggressiveness, it can also be used to distinguish more aggressive tumors from more indolent ones (García-Perdomo

et al., 2018; Pettersson et al., 2012; Yao et al., 2014; Young et al., 2012). As a diagnostic biomarker, it is very specific, but not very sensitive. In this sense, its use can be complemented by other biomarkers such as PCa antigen 3 (PCA3), which overcome the problem of sensitivity (Rigau et al., 2010; Young et al., 2012). ADAM12 as a urinary biomarker has some evidence in breast cancer, gastric cancer, bladder cancer, and small cell lung cancer. In the case of breast cancer, it can be useful for identifying women who are at risk of developing malignant disease. In gastric cancer, it allows us to discriminate between healthy individuals and cancer patients and can be used as a diagnostic biomarker, even in the early stages of the disease (Nyren-Erickson et al., 2014; Shao et al., 2014; Shimura et al., 2015). In bladder cancer and small cell lung cancer, high levels of ADAM12 are associated with several processes related to development of cancer such as proliferation, invasion, and metastasization. Some metalloproteases have also been studied. MMP2 and MMP9 can be used as prognostic biomarkers in bladder cancer. When used in combination with mucin-16 (CA125) levels, they can be indicators of ovarian cancer, especially in cases of more advanced disease (Coticchia et al., 2011). MMP7 and MMP9 levels are elevated in bladder cancer, and this increase is quite noticeable in cases of bladder cancer metastatic disease, which can be useful in the prognosis of this disease (Szarvas et al., 2011; Gogalic et al., 2015) MMP2, MMP7, and MMP9 have the potential to be used as diagnostic biomarkers for colorectal cancer (Peng et al., 2023) There is very little literature studying the relationship between the protein levels of proteases and head cancers. However, there are studies suggesting that urinary metalloproteases, in addition to playing a major role in diagnosis, can be useful in monitoring patients. Urinary KLK10 has been studied in patients with gastric cancer. This protein is significantly increased in patients with inoperable gastric cancer compared with patients with operable gastric cancer and may be predictive of cases of incurable gastric cancer (Shimura et al., 2017).

9. Conclusions

Proteases play a leading role as diagnostic and prognostic biomarkers. In recent decades, they have been extensively studied, particularly in the case of metalloproteases. MMP9 and MMP2 are among the metalloproteases with the most scientific evidence to support their potential as prognostic biomarkers in cancer. Serum MMP7, MMP9, and PSA have been shown to have prognostic predictive value in some types of cancer. In the case of saliva, the proteases with the greatest potential to be translated into clinical practice are MMP1, MMP3, MMP8, MMP9, MMP10, MMP12, MMP13, and TIMP2. In the case of urine, proteases with the potential to be used as cancer biomarkers have also been identified. These proteases are MMP2, MMP7, MMP9, ADAM12, KLK10, and TMPRSS2:ERG. When studying protease polymorphisms and their impact on cancer, the polymorphisms with the greatest influence belong to CASP-8, CASP-9, CASP-10, MMP1, MMP2, MMP7, and MMP9. There are some validated biomarkers, with PSA being the best known and most widely used biomarker in clinical practice for prostate cancer screening. Some of the challenges of using proteases as diagnostic biomarkers are related to their specificity. For instance, metalloproteases, in particular MMPs such as MMP9, play a very important role in the remodeling of the ECM which is a limiting step in cell invasion and metastasization. Nevertheless, their use as isolated

biomarkers is not very specific in diagnosis and prognosis, because many of them are altered in distinct cancer types and in other diseases. However, panels of biomarkers that include proteases that are highly related to the carcinogenesis process can overcome the problem of specificity, making it possible to translate them into clinical practice. In the future, larger cohort studies are needed to validate these biomarkers with the greatest potential to be translated into clinical practice.

Declaration of competing interest

The authors declare no conflicts of interest. All authors have read the journal's authorship agreement and policy on disclosure of potential conflicts of interest.

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CHAPTER

4

Matrix metalloproteinases and uveal melanoma

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Abbreviations

BAP1 BRCA1-associated protein 1
BRAF human gene that encodes B-Raf protein
CAM chorioallantoic membrane
CCND cyclin D
CM cutaneous melanoma
DFS disease-free survival
ECM extracellular matrix
ERK extracellular signal–regulated kinases
Fas receptors tumor necrosis factor receptor superfamily member 6
GEPIA gene expression profiling interactive analysis
GNA11 guanine nucleotide-binding protein subunit alpha-11
GNAQ guanine nucleotide-binding protein G(q) subunit alpha (Gαq)
INK4A INK4 is a family of cyclin-dependent kinase inhibitors
MEK mitogen-activated protein kinase
MMP matrix metalloproteinase
mOS median overall survival
NRAS human neuroblastoma rat sarcoma (RAS) viral oncogene homolog encoding NRAS protein
OS overall survival
PFS progression-free survival
RAF rapidly accelerated fibrosarcoma
SF3B1 splicing factor 3b subunit 1

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TCGA the cancer genome atlas
TIMP tissue inhibitor of matrix metalloproteinase
TNF receptors Tumor necrosis factor receptors
UALCAN The University of Alabama at Birmingham Cancer data analysis portal
UM Uveal melanoma

1. Introduction

Uveal melanoma (UM) is the most common intraocular cancer, but it is a rare cancer subtype. UM has emerged as a significant global risk for cancer-related mortality, with an annual identification of over 7000 new cases. The incidence rate stands at 4.3 per million in the adult population (Kivelä, 2009; Singh and Topham, 2003). The reported incidence of UM is 8 per million per year in the United States (Seddon et al., 1994), and in Australia (Vajdic et al., 2003). The mean age of UM incidence is 60 years, and males have a slightly higher predominance to females (Singh et al., 1998). Though UM is responsible for significant healthcare burden for managing the disease, the cost associated with treating UM is understudied (Crump et al., 2023). When metastasized, liver is the primary organ affected, and it may associate with a prolonged period of progression-free survival (PFS), which do not respond to any available therapeutic intervention and proved to be fatal within 12–15 months from diagnosis (Gambrelle et al., 2007; Gamel et al., 1993).

In majority of the cases, choroid is the primary affected tissue in UM, and origin of primary malignancy in either iris or ciliary body is rare (4%–6%) (Shields et al., 2012). The etiology of UM is still not understood very well. Malignant melanoma could be life-threatening, and primary site of origin could be different organs ranging from skin to eye. Primary intraocular melanoma is commonly known as UM, which is genetically different from the skin or CM. The malignancy in UM originates from choroid. Clinically, UM prevalence is being reported mostly in the Caucasians, and it has been hypothesized that having light skin and eye color are the predisposing factors in UM development (Virgili et al., 2007; Weis et al., 2006). Chronic exposure of sunlight has been identified as a major risk factor for developing UM-related malignancies. The protective role of dark pigmentation in UM has been supported by the European population-based prevalence analysis in UM where a north–south decreasing gradient of UM incidence has been reported (Virgili et al., 2007). The UV exposure–induced genetic mutation as found in CM is different from UM, and different pathways for the UV exposure–induced UM development and progression have been proposed (Dono et al., 2014; Griewank et al., 2013; Harbour et al., 2013; Horn et al., 2013; Huang et al., 2013; Koopmans et al., 2014; Luscan et al., 2015; Martin et al., 2013). Preexisting uveal nevi is thought to be the primary basis of UM development, and 5%–10% of the Caucasians have such nevi in their eyes, and it has been predicted that one out of 8845 cases may transform to UM (Singh et al., 2005).

2. Molecular basis of UM

The molecular basis of UM and its management have been extensively reviewed by Grisanti and Tura (2018). The diagnosis of UM most often takes place in the nonmetastatic phase of tumor progression and the treatment is mainly focused on managing primary tumor growth. When devising strategies for managing UM, factors such as the tumor's size and primary location, along with demographic elements such as sex, age, and visual acuity of the fellow eye, are carefully considered. Oftentimes the outcome of the management of this disease condition is not so favorable and results either loss of vision or enucleation of the eye. In the majority of cases, successful control of the primary malignant tumor is achieved; however, metastasis is a common occurrence in approximately half of UM patients, often manifesting even after several years. Gene profiling data analysis identified two types of UM with and without monosomy 3 (Tschentscher et al., 2003). So far, monosomy 3 has been established as a strong predictor for metastatic risk (Singh et al., 2012). Until now, our knowledge is limited when it comes to effectively manage metastatic UM with poor prognosis of mean survival period of <1 year upon diagnosis. Recent knowledge in this specific area also points toward the certain changes of chromosomes 3 and 8, mutations in BAP1, tumor suppressor gene, and in SF3B1, splicing factor gene. The immune checkpoint–based therapy is also gaining importance as one of the adjuvant therapies to manage the disease.

3. Cytogenetic and molecular alterations in UM

Pluripotent neural crest cells, which migrate and settle in various anatomical locations such as the epidermis, dermis, and uveal tract, have been identified as the source of the primary tumor. The melanocytes present in the uveal tract exhibit notable distinctions in developmental lineage and cytogenetic profile when compared with their counterparts in CM. UM tumors are known to show mutations in their chromosomes 1, 3, 6, and 8. Monosomy 3, the loss of chromosome 3 in the primary malignant UM tumor, has been strongly correlated with developing high risk of metastasis (Singh et al., 2012). The gain of 8q has been linked to diminished survival rates and is often observed in conjunction with monosomy 3, regarded as a subsequent event following the loss of chromosome 3. Additional mutations, such as the loss of 6q or 1p, further increase the risk of metastasis. In contrast, the gain of 6p tends to occur almost exclusively in cases without monosomy 3 and is linked to a more favorable prognosis (Aalto et al., 2001; Horsman et al., 1990; Prescher et al., 1990, 1996; Singh et al., 2009; Sisley et al., 1997; Tschentscher et al., 2000).

3.1 Oncogenic mutations in UM

It is a known phenomenon that malignancy originated due to oncogenic mutations taken place in the question cell types that lead to altered cell cycle with an end result of programmed cell death. The link of chromosome 3 in explaining UM is partly the success so far in defining the condition, but there could be other genetic contributors that might play

a significant role have not been either identified or explained. The RAF/MEK/ERK pathway and enhanced expression of cyclin D (CCND), a cell cycle regulatory protein, have been emerging as a key signaling mechanisms in melanocyte homeostasis. Phosphorylation-dependent activation of the RAF/MEK/ERK pathway results in retinoblastoma tumor suppressor gene inactivation (Onken et al., 2008), leading to tumor progression. Inactivation of INK4A gene encoding cyclin-dependent kinase inhibitor 2A has been positively correlated with an impairment in the retinoblastoma protein. The destabilization of the retinoblastoma protein ultimately enables the question cells to enter the cell cycle (Landreville et al., 2008).

The oncogenic mutation in the RAF/MEK/ERK pathway affects the genetic homeostasis of GNAQ and GNA11 in codon 29. GNAQ is recognized for its involvement in the endothelin signaling pathway, a crucial event necessary for the survival of melanocytes in the early stages of development (Van Raamsdonk et al., 2009). GNAQ activation portraying RAF/MEK/ERK signaling pathway leads transcriptional activation of CCND1 gene, which is commonly overexpressed in UM. The occurrence of GNAQ mutations has been documented in 45%–49% of biopsy samples from UM, while GNA11 mutations have been identified in 31.9% of such samples (Van Raamsdonk et al., 2010).

4. Role of extracellular matrix in cancer progression

Extracellular matrix (ECM) is known to play pivotal roles in cellular physiology and in intercellular interactions. Alterations in ECM lead to a change in cellular environment with a result of structural and morphological changes responsible for many pathological conditions (Bonnans et al., 2014). ECM dysregulation is responsible for tumor growth, cell migration, vascularization, and loss of cell adhesion (Walker et al., 2018). ECM dysregulation not only causes altered cell adhesion and migration, but it also modulates the expression of different genes responsible for cell differentiation and growth (Poltavets et al., 2018). Cellular differentiation is also widely linked to other processes including cellular homeostasis (Watt and Fujiwara, 2011), wound healing (Xue and Jackson, 2015), and cancer metastasis (Gkretsi and Stylianopoulos, 2018; Jinka et al., 2012). ECM remodeling is fine regulated by several proteins capable of hydrolyzing ECM components, leading to the pathophysiological modifications of ECM impacting many cellular processes already mentioned (Bonnans et al., 2014; Nagase and Woessner, 1999).

4.1 Matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases and their role in physiology and pathology

Matrix metalloproteinase (MMP) is a family of proteases capable of ECM degradation. MMP family in human consists of 24 proteins encoded by genes MMP 1-28. No studies so far detected MMP-4, -5, and-6 in humans. MMPs family members are zinc-dependent proteinases implicated in ECM degradation. Collagen, proteoglycans, and laminin are the

common substrates of MMPs. MMPs are typically categorized into six major subclasses based on their primary structure, substrate specificity, and cellular localization. MMP-1, -8, and -13 are collagenases, MMP-2 and -9 are gelatinases, MMP-3 and -10 are stromelysins, MMP-7, -11, and -26 are matrilysins, MMP-14, -15, -16, -17, -24, and -25 are membrane-type MMPs and MMP-12, -19, -20, -21, -23, -27, and -28 fall under other MMP subtypes (Nagase et al., 2006; Visse and Nagase, 2003). Dysregulated MMP activities have been linked to a plethora of pathological conditions (Visse and Nagase, 2003). MMPs are known to be regulated by many endogenous and exogenous molecules. Tissue inhibitors of metalloproteinase (TIMPs) are commonly known endogenous inhibitors of MMPs (Anand-Apte et al., 1996; Mannello and Gazzanelli, 2001; Rohani and Parks, 2015). MMPs and TIMPs are well known for their roles in cell growth, invasion, and tumor metastases (Nagase et al., 2006). MMPs play key roles in physiological tissue remodeling and are also implicated in a variety of pathophysiological conditions. Normal physiological processes such as embryonic development, wound healing, and apoptosis involve MMPs (Chirco et al., 2006; Lambert et al., 2004). Pathophysiological processes such as tumor cell invasion and metastasis, modulation of cell—cell adhesion, cytokine and growth factor activation, and angiogenesis (Handsley and Edwards, 2005; Stamenkovic, 2000) are known to be controlled by abnormal MMP activation. TIMPs can selectively modulate MMP activity affecting different cellular processes mentioned in this section (Chirco et al., 2006; Lambert et al., 2004).

4.2 Altered MMP/TIMP interaction and dysregulated ECM homeostasis in UM

MMP-2 has been proposed as a prognostic biomarker in UM pathology almost 25 years ago in a pilot study, and further investigation has been proposed to determine predictive value of MMP-2 expression in a larger series (Väisänen et al., 1999). To explore the potential role of MMPs, a significant study was undertaken to examine and compare the expression and activity of MMP-2 in UM cell lines cultivated either in vitro or in vivo as tumors developed in the chick embryonic chorioallantoic membrane (CAM) model (Bérubé et al., 2005). Three cell line types (SP6.5, TP31, and SP8.0) derived from primary UM tumor from three patients and one cell line derived from metastatic lesion derived from the liver of a patient diagnosed with UM have been used. Inactive form of MMP2 was detected in the vicinity of all UM cell culture models in vitro with an indication of higher expression in liver-derived H79 cell line, whereas SP8.0 cell line showed very low level of expression. The solid tumor models produced in CAM membrane showed evidence of active form of MMP-2. The UM tissue was identified with significantly higher active MMP2 gelatinolytic activity compared with the nonneoplastic CAM. Those results indicated activation of pro-MMP-2 is an important cellular event in the process of UM progression. Following this line of investigation, further research has been continued and expression and distribution of MMPs and TIMPs in human UM have been systematically studied by Lai et al. (2008). In this study, strong MMP-2 expression has been reported in tumor vasculature and in stromal cells. Heterogenous expression of MMP-1, -9, MT1-MMP, and TIMP-2 in the vasculature, and by MMP-9

expression in the extravascular matrix were detected. The expression of MMPs and TMPs point a clear role of both in tumor growth and angiogenesis.

The ECM plays a critical role in regulating tissue microenvironment and maintains cellular homeostasis. MMP-9 has been implicated pretty often in cancer development including melanoma progression. Dysregulated MAPKs and PI3K/Akt cell signaling pathways are correlated with aberrant overexpression of MMP-9. Noncoding RNAs (ncRNAs) are implicated in the aberrant synthesis of MMP-9, and other MMP-9 modulatory proteins, such as osteopontin and TIMPs. Oncological studies have provided evidence showing MMP's multifaceted role in tumor progression via ECM degradation of surrounding tissues, modulating membrane receptors and growth factors, activation of adhesion molecules, inflammatory molecules, and chemoattractive proteins (Behrendt et al., 2012; Cathcart et al., 2015; Zucker and Vacirca, 2004). Though MMP's role in CM has been widely described (Azoury and Lange, 2014; Falzone et al., 2016; Leonardi et al., 2018; Villanueva and Herlyn, 2008), not enough knowledge is available on MMP's role in UM pathology.

A handful of research has established a possible link of MMPs role with UM pathology. Latent MMP-2 and -9 expression has been positively correlated with UM metastasis (El-Shabrawi et al., 2001; Väisänen et al., 1999). MMP-9 expression is mostly found in the epithelioid mixed tumor in the aggressive form of choroidal melanomas (El-Shabrawi et al., 2001). Mixed morphology UM has shown evidence of MMP-2, -9, -13, and MT1-MMP expression (Laver, 2002). UM metastases patients expressing TIMP-1 and -2, the endogenous inhibitors of MMP-2 and -9 have shown a higher survival rate (El-Shabrawi et al., 2001). Gene microarray data indicate downregulated TIMP-3 mRNA expression in class 2 UM and described to be associated with poor prognosis (Singh et al., 2007), and expression of TIMP-3 was not found in 6 out of 10 UM with monosomy 3 (Nareyeck et al., 2005). Interestingly, TIMP-3 gene expression has been reported to be downregulated by 80% and controlled by DNA methylation in metastatic primary UM cells. That indicates TIMP-3 as a progression marker (Van der Velden et al., 2003) though the association of TIMP3 mRNA expression and histopathological linkage of metastatic UM has not been clinically established (Nareyeck et al., 2005; Van der Velden et al., 2003). The regiospecific and topographical expression of MMP/TIMPs and their differential distribution in stroma and tumor cells and in the vasculature has not been properly described so far in UM. MMPs and TIMPs have been emerging as prognostic or progression biomarkers in UM.

UM metastases are being diagnosed almost 10 years PFS after successfully treating the primary malignancy (Gamel et al., 1993). So far the UM management strategies involving available therapeutic options have proven to be unsuccessful, and several studies performed in this area provided evidence showing somatic and genetic changes in the tumor is strongly associated with the metastasis.

Analysis of the global mRNA expression studies have identified some genes that are differentially expressed between tumors having monosomy 3 and disomy 3 (Tschentscher et al., 2003). Thus, altered protein expression due to such genetic mutations could be the missing elements closing the gap of genetic contributor to the clinical manifestations of the UM.

Among such genes TIMP-3, a 24-kDa protein has been identified with meaningful effect(s) on UM development and progression (Yu et al., 2000). In a cell culture model consisting of two UM cell lines derived from metastatic lesions, hypermethylation of the TIMP-3 gene has been reported to be negatively correlated with UM metastases in comparison with the primary UM cell line derived from the same patient (Van der Velden et al., 2003). This finding strongly supports altered TIMP-3 expression may be important in the development of UM metastasis.

TIMP-3 is specifically known as an inhibitor of angiogenesis (Qi et al., 2003). Since ECM remodeling and angiogenesis both consist of integral pathways for the tumorigenesis, loss of TIMP-3 gene expression has been positively correlated with progression of UM metastasis. However, due to multimodal effect of TIMP-3 gene expression, the protective role of TIMP-3 as a downregulator of UM metastasis has not been equivocally established (Guo et al., 2001). TIMP-3 has been implicated to trigger apoptosis in CM by protecting the death signaling receptors including Fas and the TNF receptors from degradation and as a consequence has negative effect on tumor progression (Ahonen et al., 2003).

An important study (Nareyeck et al., 2005) conducted about 15 yrs ago where TIMP-3 expression in 19 UM cases was examined and compared the results with histopathological and genetic features. The expression of TIMP3 mRNA as determined by microarray analysis was identified in association with the chromosome 3 status of the tumor. Six tumors with monosomy 3 have been identified with no TIMP3 gene expression. This data is critical and indicated a possible protective role of TIMP3 as a tumor suppressor element in UM.

In a recent study (Wang et al., 2021), the prognostic implications of different MMPs expression have been described. The study presented survival plots of MMPs in the UM obtained from the cancer genome atlas (TCGA) dataset from UALCAN, a portal for facilitating tumor subgroup gene expression and survival analyses. Effect of MMPs on patients' OS is presented in Fig. 4.1.

Fig. 4.1 shows the prognostic value of 9 MMPs family members in UM patients. The OS survival curves from patients with high and low MMP expression. Results analyzed by the online tools from the University of Alabama at Birmingham Cancer data analysis portal.

The increased mRNA expression data of MMP-1, -2, -9, and -16 has been shown to be significantly associated with the UM prognosis and poor OS, while those of MMP-14, 15, -17, and =24 were identified not related to OS in UM.

The study also used dataset obtained from Gene Expression Profiling Interactive Analysis (GEPIA) to compare the mRNA expression of MMPs to the OS and DFS of UM patients. The summary data of this study for DFS and OS curves are presented in Figs. 4.2 and 4.3. Significant associations between MMP-1, -2, -9, -11, and-16 and OS have been observed, which mirrored the results of TCGA data analysis. Interestingly, significant association between MMP-1, -2, -9, -11, -14, and -16 and DFS was observed in this pioneering study. These results point toward MMP-1, -2, -9, and-16 expression levels as a predictive tool and potential prognostic biomarkers for the UM patients survival outcome.

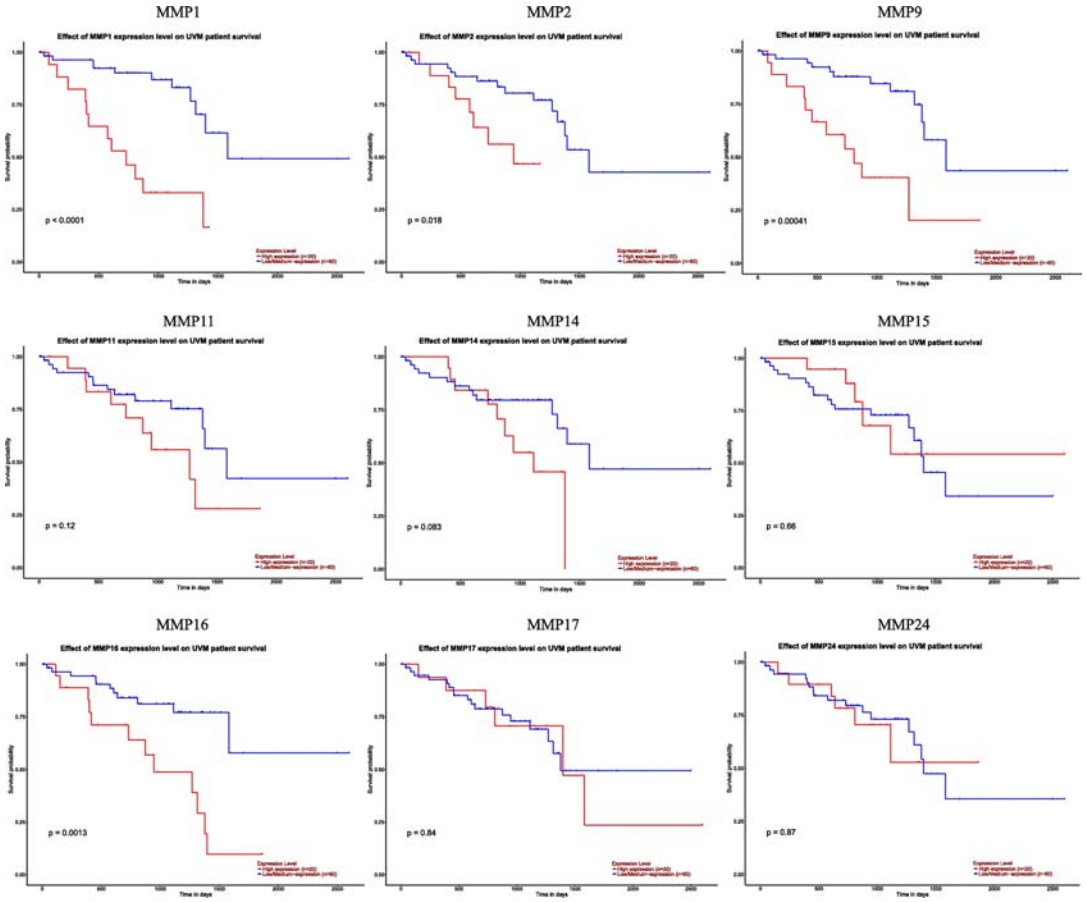

FIGURE 4.1 The OS survival curves comparing patients with high and low MMPs family member expressions. Data analyzed by the University of Alabama at Birmingham Cancer data analysis portal online tool. The prognostic value of 9 MMPs family members in UM patients (UALCAN). The OS survival curves comparing patients with high (*red*) and low (*blue*) MMPs family member expressions were plotted. The threshold of *P* value is .05. *Taken from Wang et al. (2021).*

Fig. 4.2 shows the prognostic value of 9 MMPs family members in UVM patients and the disease-free survival curves from patients with high and low MMP expression. Results analyzed using gene expression profiling interactive analysis.

Fig. 4.3 shows the prognostic value of 9 MMPs family members in UM patients and the overall survival curves from patients with high and low MMP expression. Results analyzed using gene expression profiling interactive analysis.

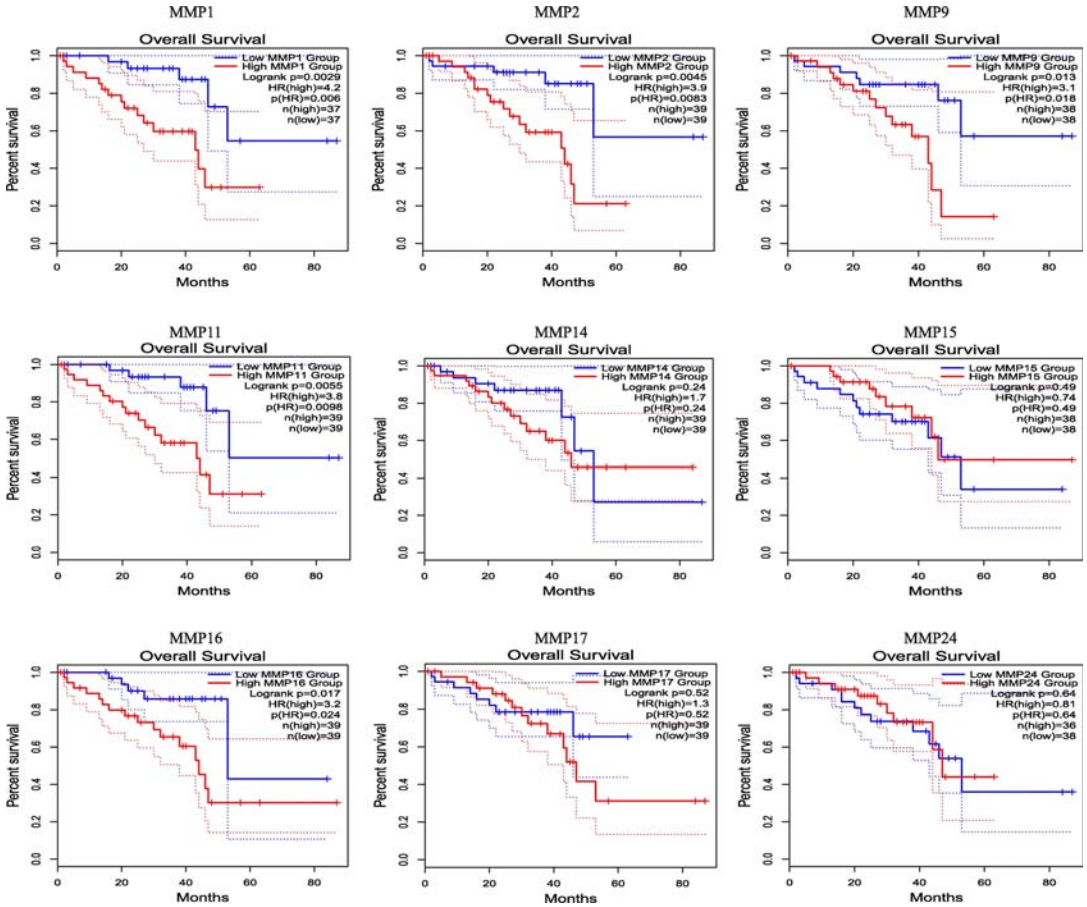

FIGURE 4.2 The DFS survival curves comparing patients with high and low MMPs family member expressions. Data analyzed by the Gene Expression Profiling Interactive Analysis (GEPIA) online tool. The prognostic value of 9 MMPs family members in UVM patients (GEPIA). The DFS survival curves comparing patients with high (*red*) and low (*blue*) MMPs family member expressions were plotted. The threshold of *P* value is .05. *Taken from Wang et al. (2021)*.

5. Conclusions and future directions

Immunological therapies in UM management have been gradually gaining importance in several types of cancers including UM. CM mainly involves mutations in NRAS or BRAF, whereas UM is mostly associated with GNA11 or GNAQ mutations. Until now, there has been no standard therapy available to manage the UM for its prevention or treatment of metastases. Chemotherapy and targeted therapy do not provide adequate positive data to be accepted as standard management process due to least responses in patients with metastatic UM. The median survival time is only 4–5 months since metastasis is detected.

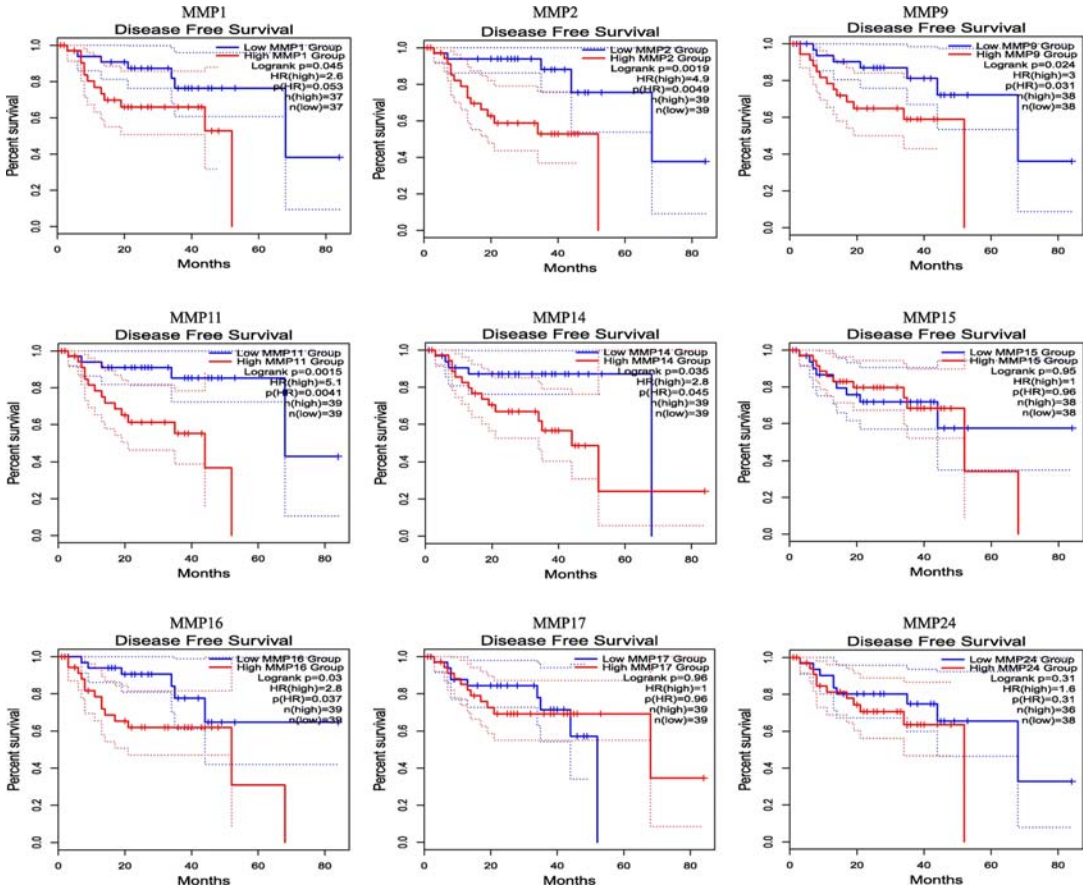

FIGURE 4.3 The overall survival curves comparing patients with high and low MMPs family member expressions. Data analyzed by the Gene Expression Profiling Interactive Analysis (GEPIA) online tool. The prognostic value of 9 MMPs family members in UM patients (GEPIA). The OS survival curves comparing patients with high (*red*) and low (*blue*) MMPs family member expressions were plotted. The threshold of *P* value is .05. *Taken from Wang et al. (2021)*

Immunotherapy agents, such as immune checkpoint inhibitors that are proven to have promising results in CM, failed to provide a positive outcome in UM management. Identifying newer immune checkpoint(s) and therapeutic targeting are appeared to be the future strategies for the disease management. Recent advances and challenges in UM immunotherapy have been reviewed in detailed by Fu et al. (2022).

Cancer vaccines did not provide favorable results. Recent clinical trial data involving tebentafusp (KIMMTRAK), a bispecific monoclonal antibody considered as first-in-class immunotherapy agent containing an engineered T cell receptor molecule that targets a gp100 epitope of human leukocyte antigen (Howlett et al., 2023). Tebentafusp provided promising antitumor effects and is the first FDA-approved immune therapy showing

significantly extended median overall survival (mOS) by 6 months versus other immune checkpoint inhibition (Nathan et al., 2021). Tebentafusp does have limited specificity toward targeting cancer cells, and normal melanocytes targeting has also been reported (Boudousquie et al., 2017; Middleton et al., 2020). Oncolytic viruses and small molecule inhibitors are also appearing as strong contenders as possible therapeutic intervention for clinical management of UM.

Although there has been a big gap in the knowledge of understanding the molecular basis of UM development, researchers across the globe are now devoting significant effort to uncover the molecular and genetic pathways involved to explain and understand the disease condition to develop therapeutic avenues to better manage the disease. MMPs role has also been explained pretty well in relation to different cancer metastasis including the cell cycle pathways involved in the UM metastasis. Many MMPs are correlated well with the infiltrating immune cells (Pan et al., 2023; Zeng et al., 2022) in several types of cancer progression. There has been strong evidence for MMP-1 and MMP-9 (Lamas et al., 2022; Wang et al., 2021) to be clinically important to provide as prognostic biomarkers and as therapeutic targets for UM management. However, additional studies and large-scale clinical trials are necessary to validate these observations.

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CHAPTER

5

Proteases in DNA damage, repair, and radioresistance: Cellular and molecular aspects

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1. Introduction

A protease is an enzyme that catalyzes the process of proteolysis and plays critical roles in several biological processes and is associated to many pathological conditions and disorders. Extracellular proteases are well known for their function in cancer development and progression, particularly in the stimulation of cell invasion, angiogenesis, migration, and metastasis mediated through extracellular matrix remodeling. The major proteolytic enzymes promoting cancer include serine proteases, plasmin, plasminogen activators, and MMPs. Furthermore, a trypsin-like protease known as tumor-associated trypsinogen (TAT) has been found in many malignancies, and it produces a metastatic phenotype in cancer cells (Kos, 2022).

Proteases are known to regulate DNA damage, DNA repair, and cancer therapy resistance. Cancer is a complex and multifaceted disease that occurs due to the accumulation of genetic mutation that disrupts the delicate balance of cell growth and cell death. Reduced expression of these genes made cells more susceptible to radiotherapy, as seen by significantly decreased cell survival, increased apoptosis, and autophagy (Niland et al., 2022).

In this chapter, we are focusing on the molecular mechanism of proteases involved in DNA damage, repair, and radioresistance.

2. Role of proteases in DNA damage, response, and repair

Organisms respond to DNA damage caused by endogenous and exogenous agents. Endogenous DNA damage is caused primarily by cellular metabolism, ROS, gene rearrangement

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error, and DNA replication error. Other processes such as hydrolysis, oxidation, alkylation, and deamination also induce DNA damage. External DNA damaging agents include mainly physical agents such as ionizing and ultraviolet radiation, and other chemical agents including therapeutics, carcinogenic, and genotoxic substances. The DNA damage if not repaired is lethal and can result in apoptosis. On other hand, aberrant or error-prone DNA repair can result in genomic instability culminating in oncogenesis or cellular senescence. Such changes have significant impact on the organism's growth and aging process, immunocompetency, and neurological functions. DNA repair is the cellular defense mechanism against DNA damage and mutations. DNA repair mechanism is tightly regulated to safeguard the genome from various types of damage, which include single- and double-stranded breaks, DNA cross-links and base modification, etc. Several proteins and proteases are involved in the coordination of DNA repair process. Proteases are essential for DNA repair process because it plays an important role by cleaving and processing of proteins participating in DNA repair pathways. Nuclear proteases are involved in chromatin remodeling, processing and activation of transcription factor, and regulation of gene expression. Any changes in these protease activation or expression lead to immunological disorders, cancer, and cell death. Proteases help to maintain the structural integrity of the nucleus (Frolova et al., 2023).

The major proteases that are involved in DNA damage repair and maintain genomic stability are described in the following.

2.1 Protein cross-link proteases (DPC proteases)

DNA–protein cross-links (DPCs) are very harmful DNA lesions generated by covalent cross-linking of proteins and DNA. DPCs can be caused by a variety of factors, including exposure to radiation, chemotherapy drugs, and certain chemicals. DPCs block DNA replication, repair, and transcriptions. DPC proteases cleave the protein component of DPCs, thus safeguarding genomic integrity. Dysfunction of DPC proteases causes genomic instability, cell cycle arrest, accelerated aging, and cancer susceptibility. Wss1 and SPRTN, DPC proteases, perform critical roles in DNA repair and genome stability. They participate in the breakdown of DPCs, which are extremely toxic DNA adducts that disrupt DNA replication (Reinking et al., 2020).

2.1.1 Wss1 protease

Wss1 is required for cell survival following UV irradiation. Upon UV-induced DNA damage, DNA damage response is initiated. This facilitates the sumoylation of repair proteins. The addition of these sumo tags facilitates the recruitment of a ternary complex comprising of Wss1-Cdc48 AAA ATPase-Doa1 to the site of sumo-tagged DNA damage. This activates Wss1 induces self-cleavage of metalloprotease and proteolysis of associated proteins (Balakirev et al., 2015).

2.1.2 SPRTN (Spartan)

SPRTN is a protease that is known as a DNA-dependent protease, which is only active when attached to DNA. SPRTN is a mammalian protease that is essential for the breakdown of DPCs. Cells that lack SPRTN expression or have SPRTN mutations are vulnerable to therapies that produce covalent DPC. The protease activity of SPRTN removes DPCs, by

degrading the cross-linking proteins from DNA–protein covalent complex, protecting cells from DNA damage. Proteases of the SPRTN family thus play a pivotal role in maintaining genome stability, normal cell cycle progression, cell viability, and ensuring tumor suppression through eradicating endogenous DPCs, which might possess a threat to chromatin remodeling (Ruggiano et al., 2021).

2.1.3 CTLA-4

CTLA-4 participates in the DNA damage response and regulates immune activity under high-stress situations. CTLA-4's are involved in indirect activation of the ataxia–telangiectasia mutated (ATM) protein by binding to the ATM inhibitor protein phosphatase 2A.This activation of ATM exacerbates the DNA damage response and causes T cells to die. In eukaryotes, DNA damage signaling kinases play an essential role in coordinating the DDR proteins, and it can directly regulate DNA repair factors. In response to DNA damage, CTLA4 enables the initiation of apoptosis especially in T cells. During DNA damage response, CTLA-4 interacts with protein phosphatase 2A (PP2A) and prevents its translocation to nucleus. This prevents PPA2 from inhibiting the function of ATM, a nuclear protein that initiates DNA damage response. Thus CTLA4 enhances ATM-induced DNA damage response, leading to apoptosis (Yan et al., 2022).

2.1.4 DVC1 (DNA damage protein targeting VCP)

DVC1 (DNA damage protein targeting VCP) is a member of the Hsp100/Clp family of proteases that plays a major role in integrating cell cycle regulation and DNA repair. DVC1 is a homolog of the yeast protein Wss1, which is a well-characterized protease with activity against DPCs. DVC1 is also able to cleave DPCs, but its activity is less robust than that of Wss1. In addition to its protease activity, DVC1 is also shown to have ubiquitin ligase activity. The protease activity of DVC1 is essential for its role in repair process of DNA allowing the elimination of cross-links, thus facilitating DNA repair. DVC1 is a promising therapeutic target for several diseases, including cancer and neurodegenerative diseases. Mutations in DVC1 have been linked to an increased risk of these diseases, and inhibition of DVC1 protease activity has been shown to have anticancer and neuroprotective effects (Stingele et al., 2017).

2.1.5 ADAM 10

ADAM10 belongs to the ADAM (a disintegrin and metalloprotease) protease family, which disintegrates cell surface proteins, coordinating several biological processes such as cell to cell and autocrine signaling, cell adhesion, cell migration, and cell division. Dysregulation in its expression and activity have been linked to a variety of pathological conditions such as asthma, arthritis, neurodegenerative diseases, atherosclerosis, and cancer (Rossello et al., 2020). ADAM10 is a significant regulator of CD46 expression during cellular apoptosis. It enhances apoptosis during UV-induced damage by regulating the shedding of CD46 ectodomain from the vesicles of apoptotic cells aggravating apoptosis.

2.2 Aspartic proteases

Aspartic proteases are involved in DNA repair pathways, including double-stranded DNA repair pathways such as homologous recombination (HR) and nonhomologous end-joining

(NHEJ). In NHEJ, aspartic proteases degrade damaged DNA ends, allowing for the ligation of DNA fragments. In HR, aspartic proteases degrade proteins involved in the formation of Holliday junctions, which are intermediate structures in homologous DNA recombination (Serbyn et al., 2020).

2.2.1 DDI1

DNA damage-inducible I (DDI1) is an aspartic protease that gets activated in response to DNA damage. DDI1 is participated in a number of cellular processes, including DNA repair, ubiquitination, and also in degradation of protein. DDI1 is essential for the DNA repair when the damage is induced by UV radiation, ionizing radiation, and other environmental factors. Cells that lack DDI1 are more vulnerable to DNA damage and more prone to acquire mutations. DDI1 is also involved in protein ubiquitination, a process that marks proteins for destruction. The ubiquitin ligase Rsp5 interacts with DDI1, and this association is required for the ubiquitination of numerous proteins, including the DNA repair protein Rad51 and possible therapeutic target for cancer therapy. DDI1 inhibitors have been proven in studies to sensitize cancer cells to chemotherapy medicines and radiation treatment. DDI1 inhibitors may potentially be beneficial in the treatment of other disorders induced by DNA damage, such as neurodegenerative diseases (Serbyn et al., 2020).

2.2.2 DNA damage-inducible 2

DNA damage-inducible 2 (DDI2) is induced and activated in response to the DNA damage, which preserves the DNA integrity by repairing damaged DNA and regulating cellular stress responses. DDI2 is involved in the repair of several forms of DNA damage, including UV radiation−induced DNA damage, ionizing radiation-induced DNA damage, and chemotherapy-induced DNA damage. DDI2 interacts with several DNA repair proteins, including XPC, RAD51, and BRCA1, to aid in the repair of damaged DNA (Koizumi et al., 2016; Lehrbach and Ruvkun, 2016).

2.3 Proteasome

The proteasome is a large multiprotein complex that degrades proteins that are tagged with ubiquitin. It can also be triggered as a result of DNA damage, where it has the ability to degrade nucleases and DNA polymerases. Protease activity is tightly regulated in cells to prevent excessive DNA damage. However, mutations or other changes in protease activity can contribute to cancer and other diseases. They can be activated in response to DNA damage to cleave other proteins involved in DNA repair, cell cycle progression, histones and chromatin remodeling, transcription, and apoptosis. Proteolysis of these proteins can lead to a variety of cellular responses, including cell cycle arrest, apoptosis, or alteration in cell cycle leading to cellular senescence, thus helping the genomic integrity to be maintained in a regulated manner. Proteasome inhibitors have been explored as potential anticancer agents by disrupting DNA repair mechanisms and promoting apoptosis (Manasanch and Orlowski, 2017).

2.4 Ubiquitin-specific proteases

Abnormal protein ubiquitylation regulation is associated to oncogenesis and disease progression and other pathologies such as immune disorders, neuromuscular disorders,

muscular dystrophies, and infection. As ubiquitin-specific proteases (USPs) target various proteins involved in regulating cell homeostasis, tumor suppressor proteins, and oncoproteins, they may be considered as target protein for regulating and inhibiting abnormal protease activities, leading to disorders and disease progressions. UPS regulates DNA repair and other several biological activities as well. In mammalian global genome nucleotide excision repair (GG-NER), activation of the DDB2-associated ubiquitin ligase in response to UV-induced DNA damage is necessary for effective identification of lesions. The UPS is responsible for the degradation of several proteins involved in DNA repair, including those implicated in DNA double-strand break repair (Chen et al., 2021).

2.4.1 USP7

It is a cysteine protease that specifically cleaves ubiquitin from ubiquitinated proteins. USP7 plays a crucial role in regulating these processes by removing ubiquitin and altering the fate of the modified proteins. DNA damage response facilitates the recruited of USP7 to sites of DNA damage via interactions with other DDR proteins, such as RNF168 and MDC1. USP7 interacts with MRN-MDC1 complex and recruits the downstream protein p53 to the site of damage. USP7 mediates the deubiquitination of a variety of proteins involved in the DDR, including H2AX, BRCA1, RAD51, p53, and MDC1. USP7 enhances the DNA repair by stabilizing MRN-MDC complex, during DNA double-stranded break repair. USP7 mediated deubiquitination and MDC1 stabilization, promoting oncogenesis and progression and resistance to DNA-targeted therapies (Spiliotopoulos et al., 2019).

2.4.2 USP11

Human ubiquitin-specific protease 11 is recognized for its role in DSB repair by homologous recombination (HR). Knockdown of this protease causes the spontaneous activation of the DDR proteins and sensitivity to genotoxic stress agents and ionizing radiation. USP11 interacts with ubiquitinated DNA repair proteins, such as RAD51 and BRCA2, facilitating their proper functioning and repair of damaged DNA. This allows these proteins to arrest the cell cycle and prevent it from dividing, until damaged DNA has been repaired (Jacq et al., 2013; Orthwein et al., 2015). There are two main types of DUBs: cysteine proteases and metalloproteases. Cysteine proteases use a cysteine residue in their active site to cleave the isopeptide bond between ubiquitin and the target protein. Metalloproteases use a metal ion, such as zinc, to cleave the isopeptide bond (Wei et al., 2015).

2.5 SUMO proteases

Small ubiquitin-like modifier proteases (SUMO) proteases are enzymes that remove SUMO groups from proteins. SUMOylation is a posttranslational modification that can regulate protein activity, stability, and localization. SUMO proteases can be activated in response to DNA damage, where they can remove SUMO groups from proteins involved in DNA repair or cell cycle progression. Over thousands of SUMO substrates and SUMOylated lysines are involved in transcriptional regulation, chromatin modification, and DNA damage response. In response to a DNA damage caused by endogenous or exogenous

response, a number of proteins were found to be upregulated and downregulated in SUMOylation, indicating the importance of SUMO in the DDR. SUMOylation of histone demethylases was found to regulate their activity in response to DNA damage, resulting in transcriptional repression. SUMO coordinates several nuclear processes, including transcription, chromatin organization, and DDR, in response to DNA damage (Hendriks and Vertegaal, 2015).

2.6 Calpains

Calpains are calcium-dependent proteases that are also activated in response to DNA damage. They can cleave a range of proteins that are involved in DNA repair, including DNA repair enzymes, histones, and chromatin remodeling proteins. On the cellular level, inhibition of pharmacological calpain increases the sensitivities of primary glioblastoma cells toward the drug temozolomide, which is a common oral alkylating agent used to treat glioblastoma. The genetic knockdown of calpain-2 in U251 cells helps to enhance the cleavage of caspase-3 and susceptibility to neocarzinostatin, which rapidly promotes the breakage of DNA strand (Camins et al., 2006).

2.7 Caspases

Caspases are a family of proteases that play an important role in programmed cell death called apoptosis. They can also be activated in response to DNA damage, where they cleave other proteins involved in DNA repair or cell cycle progression. Caspases regulate DDR, thus helping in restoring genomic stability by eliminating faulty cells. Caspases activation and apoptosis are usually induced by DNA damage through the intrinsic pathway. This pathway is initiated when the members of the B cell lymphoma-2 (BCL2) protein family of anti- and proapoptotic proteins are activated. However, they are also involved in DNA repair by cleaving specific repair-related proteins and also involved in augmenting DNA damage in the absence of cell death, indicating their role in DNA damage response (DDR) that is different from their role in apoptosis (Lopez and Bouchier-Hayes, 2022).

2.8 Metalloprotease FAN1

FAN1 is involved in DNA interstrand cross-link repair, which is an important pathway involved in the regulation of genomic integrity. These proteases cleave the DNA at a particular site of the interstrand cross-link, allowing for further processing and repair. Mutations in the FAN1 gene are associated with Fanconi anemia, emphasizing the importance of these proteases in genomic regulation (Smogorzewska et al., 2010).

2.9 Caspase-activated DNase

Caspase-activated DNase (CAD) is primarily involved in the apoptosis process but is also involved in the repair process of DNA by cleaving and processing DNA during apoptotic

DNA fragmentation. This can thus influence DNA repair by generating double-stranded breaks (Widlak and Garrard, 2005).

2.10 FtsH protease

It is an ATP-dependent protease. FtsH is a membrane-anchored protease with an N-terminal transmembrane region and a C-terminal cytosolic region that contains AAA-ATPase and Zn^{2+} metalloprotease domains. It plays a critical role in membrane proteins integrity. It is involved in the degradation of misfolded and damaged membrane proteins, and it also plays a role in the assembly and disassembly of membrane protein complexes. FtsH degrades a collection of short-lived proteins, permitting cellular control at the protein stability level. FtsH also degrades some misassembled membrane proteins, which helps to sustain their quality. The involvement of these proteases in cancer DNA repair mechanisms underscores the complicated nature of maintaining genomic stability. Dysregulation of these proteases can lead to impaired DNA repair, accumulation of DNA damage, and increased risk for cancer development. Targeting these proteases has shown therapeutic potential in cancer treatment (Ito and Akiyama, 2005).

3. Proteases in radioresistance

Radioresistance is the ability of cells to survive exposure to ionizing radiation. Proteases play a role in radioresistance by removing damaged proteins and proteins that are no longer needed. This allows the cell to repair its DNA and to survive the radiation exposure. Radioresistance is a key factor in radiation failure and poor prognosis in tumor patients. Following radiotherapy, the activity of several metabolic pathways changes significantly, which may result in radiation resistance. Ionizing radiation stimulates rapid and persistent remodeling of extracellular matrix (ECM) through the activation of proteases and growth factors, as well as in response to chronic formation of reactive oxygen species (ROS). Certain proteases may contribute to radioresistance by increasing the DNA repair mechanism. The following is the list of some proteases that have been implicated in radioresistance.

3.1 USP 14

USP14 plays an important role in the progression of tumor radio resistance. USP14 upregulated in oral squamous cell carcinoma. It confers resistance to radiation mainly by suppressing autophagy in oral squamous cell carcinoma. Thus, its high expression results in poor prognosis of oral squamous cell carcinoma (OSCC). Thus, inhibiting the function of USP14 can improve sensitivity of OSCC cells to ionizing radiation treatment through generating apoptosis. In IR-treated OSCC cells, USP14 knockdown promoted autophagy, which contributed to the development of DNA damage and the inhibition of cell proliferation (Xie and Xu, 2020).

3.2 ClpPX protease

This is an ATP-dependent mitochondrial serine protease that aids in mitochondrial protein quality control by proteolytic digestion of misfolded or damaged proteins, enabling the normal metabolic activity to continue. ClpXP is overexpressed in hematologic malignancies and solid tumors and contributes toward treatment resistance and disease progression. Moreover, both ClpXP inhibition and hyperactivation decrease respiratory chain function and promote cell death in cancer cells (Fu et al., 2011; Nouri et al., 2020).

3.3 Lon protease

Lon protease is generally considered as an ATP-dependent mitochondrial protein, which ensures proper functioning of mitochondria when challenged with exogenous and endogenous stress. The subcellular location of these proteases is generally mitochondrial matrix; however, its nuclear and nucleoid translocation is observed, where it maintains mtDNA integrity. Upregulated expression and activation of Lon protease leads to cancer cell invasion, migration, and metastasis, contributing to disease progression (Quiros et al., 2014) (Table 5.1).

4. Conclusion and future perspective

Protease plays a prominent role in DNA damage response, repair, and apoptosis. As represented in Fig. 5.1, upon encounter of the cells with endogenous and exogenous damaging agents, DNA might get damaged. Proteases play an important role in damage response through cell cycle check point activation arresting the cells at G2/M phase. The arrested cells undergo repair if damage is rectifiable. In case of extensive damage, the cells undergo apoptosis. Proteases play a prominent role in repair and apoptosis as well. Understanding the involvement of proteases in DNA damage, repair, and resistance to

TABLE 5.1 Proteases in Radioresistance and mechanism of action.

Sl No.	Proteases involved in radioresistance	Mechanism of action	References
1	USP 14	USP14 upregulated in oral squamous cell carcinoma tissues and cell lines and progression of tumor radioresistance.	Xie and Xu (2020)
2	ClpPX protease	Increased ClpP expression is correlated with poor prognosis and lower metastasis-free survival in patients with breast adenocarcinoma, melanoma, and lung adenocarcinoma.	Feng et al. (2021)
3	Lon protease	High expression of Lon protease promotes cancer cell proliferation and invasion and the capability to migrate and form metastasis.	Quiros et al. (2014)

treatment will help lead the development of new cancer therapies. Although the therapeutic importance of detecting protease activity in vivo is well acknowledged, it is relatively difficult to obtain (Yang et al., 2009). Extracellular proteases are involved in a range of physiological and pathological processes, including tumor cell metastasis. As a result, they have emerged as ideal molecular targets for newly discovered therapies, emphasizing the necessity of knowing the molecular processes that regulate their expression and function (Park et al., 2020).

Fig. 5.1 shows proteases associated in DNA damage response. Organisms respond to DNA damage induced by various reasons such as ROS, cellular metabolic process, or gene rearrangement.

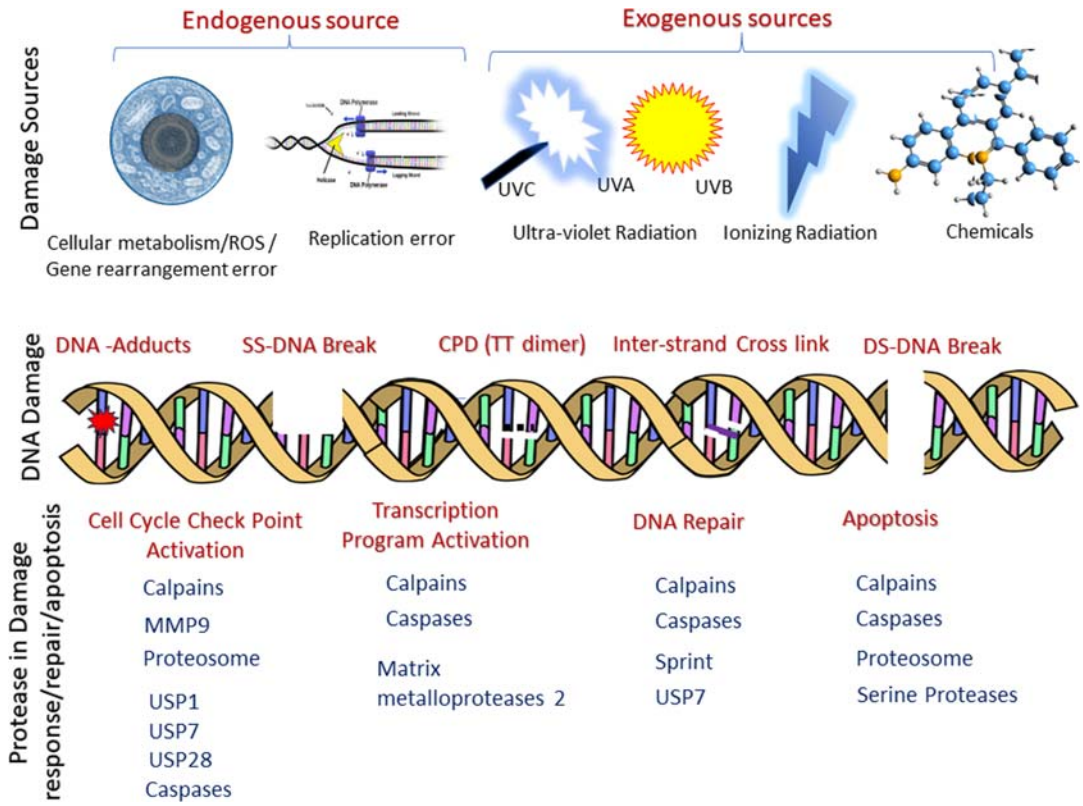

FIGURE 5.1 Proteases involved in DNA damage response. DNA damage is caused by a variety of sources. Organisms respond to DNA damage caused by endogenous factors such as cellular metabolic processes/ROS/gene rearrangement error, and replication error and exogenous factors such as UV radiation, ionizing radiation, and other chemical agents. The cellular response to damage may involve activation of a cell cycle checkpoint, initiation of transcriptional programs, execution of DNA repair, or when the damage is severe, initiation of apoptosis and several proteases are involved in these process.

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CHAPTER

6

Radiotherapy, proteases and gastric cancer: A complex interplay

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1. Introduction

Gastric cancer (GC) poses a significant global health threat, with its incidence and mortality rates varying across different regions of the world (Sung et al., 2021). Emerging as a result of uncontrolled cell proliferation within the stomach lining, GC manifests in various pathological forms, predominantly categorized into diffuse-type adenocarcinoma and intestinal-type adenocarcinoma. These classifications often correspond to distinct etiological factors (Correa, 1995). The heritable mutations in the CDH1 gene are associated with diffuse-type adenocarcinoma, while persistent colonization of *Helicobacter pylori* stands out as a major causative factor for intestinal-type adenocarcinoma (Plummer et al., 2015). The intricate cellular architecture of the gastric mucosa, with its specialized layers and cell types, underscores the complexity of maintaining gastric epithelial health and integrity. Critical to this function are the adhesive cell–cell junctions, including desmosomes, adherens junctions, and tight junctions, which regulate barrier function and tissue integrity. Dysregulation of these junctions, often stemming from alterations in transmembrane proteins, has been implicated in increased epithelial permeability, a hallmark of gastric carcinogenesis (Bernegger et al., 2022). Proteases, a diverse group of enzymes involved in protein degradation, emerge as key players in the multifaceted landscape of GC development and progression. Originating from both the host and the bacterial pathogen *H. pylori*, proteases contribute to a myriad of processes, including extracellular matrix (ECM) remodeling, inflammation, and metastasis (Sitarz et al., 2018). Their intricate interplay within the gastric microenvironment highlights the dynamic nature of GC pathogenesis. Understanding the intricate interplay between proteases and GC pathogenesis holds immense therapeutic potential. Insights into the role of host and bacterial proteases not only shed light on disease mechanisms but also offer

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avenues for targeted therapeutic interventions. Moreover, exploring the impact of radiotherapy (RT) on protease activity provides valuable insights into potential treatment modalities and highlights the need for comprehensive approaches in GC management (Kam and Banati, 2013; Fachal et al., 2014). In this chapter, we delve into the multifaceted roles of proteases in GC, exploring their contributions to inflammation, tissue remodeling, and disease progression. Additionally, we discuss the effects of RT on protease activity and its implications for therapeutic strategies in GC management.

2. Gastric cancer

The stomach, which is responsible for food breakdown and nutritional absorption, is prone to cancer development. Gastric cancer (GC), ranked as the fifth most common cancer worldwide, results from an uncontrolled cell proliferation within the stomach lining and is a serious global health hazard. According to GLOBOCAN, over 1.1 million new GC cases have been diagnosed with a mortality of 800,000 accounting for 7.7% of all cancer-related deaths in 2020. The incidence and mortality rates vary greatly by geographical regions (Sung et al., 2021). Eastern Asia has the highest burden of GC incidence and over half of all global deaths (Ilic and Ilic, 2022).

2.1 Stomach architecture and function

The stomach can be broadly divided into four distinct regions: the cardia, fundus, body, and pylorus. Each region possesses a unique histological makeup within the four main tissue layers that constitute the entire stomach wall. The outermost layer, the serosa, is a smooth, glistening membrane formed by mesothelium and connective tissue. It facilitates smooth movement within the abdominal cavity. Beneath lies the muscularis externa, a robust layer of smooth muscle responsible for the characteristic churning contractions that break down food. This layer is further subdivided into inner circular and outer longitudinal fibers, allowing for efficient mixing and propulsion of stomach contents. Inwardly, the submucosa acts as a supportive scaffold, harboring blood vessels, nerves, and lymphatics. Finally, the innermost layer, the mucosa, is the centre of digestive activity. Here, gastric pits delve into the epithelium, leading to gastric glands that secrete digestive juices. It acts as an effective barrier against the interaction of exogenous molecules with the acidic environment of the gastric lumen. It is divided into a number of different layers, and each layer is made up of specialized cell types with specific functional characteristics to preserve the health of the gastric epithelium (Bansil et al., 2013). Cell–cell interactions are essential to enable barrier function and maintain tissue integrity of the stomach epithelium. This is successfully achieved by a variety of adhesive cell–cell junctions, including desmosomes, adherens junctions, and tight junctions. Dysregulation of transmembrane proteins supporting the cell–cell junctions is well known to increase the epithelial permeability (Wei and Huang, 2013).

GC can develop in any region of the stomach; it mostly appears as two pathological variants. The diffuse-type adenocarcinoma is reported to be associated with a heritable loss-of-function mutation of the E-cadherin gene CDH1, while the main cause of intestinal type

adenocarcinoma is attributed to persistent colonization of the human pathogen *H. pylori* (Correa, 1995). Based on anatomical sites, noncardia gastric cancer (NCGC) originates in the mid or lower parts of the stomach while the cardia gastric cancer (CGC) arises in the uppermost section of the stomach, near the esophageal junction. Although factors, such as stress and alcohol consumption, are known as potential contributor in development of chronic gastritis and its further transformation into GC, but nearly 89% of NCGCs have been linked to *H. pylori* infection (Plummer et al., 2015). Regulation of transmembrane proteins has often been linked to invasion and migration of cancer cells. Cleavage events in the junction proteins have been linked to a decrease in intercellular adhesion, disturbed cell polarity, tight junction dysregulation, increased permeability, tumor cell invasion, and metastasis in cases of GC. Desmosomal adhesion disruption due to dysregulation of desmosomal proteins, such as desmogleins and desmocollins, can aid in the detachment and migration of GC cells to other sites (Kam and Banati, 2013; Fachal et al., 2014)

It is reported that protease originating from both host and bacteria contributes to development of chronic gastritis and its further transition to GC. Proteases are reported to be involved in all the events such as colonization, epithelial inflammation, and barrier disruption in tissue transformation, deregulation of cell proliferation, cell death, and subsequent metastasis of cancerous cells (Bernegger et al., 2022). An interplay between bacterial and host proteases is well known to contribute significantly in bacterial colonization. Further in the subsequent sections, we will discuss the types of proteases and their involvement in GC development as well as the contribution of *H. pylori* in cancer progression.

3. Proteases and its classification

Proteases, synonymous with peptidases or proteinases, constitute a ubiquitous class of enzymes with a pivotal role in diverse physiological and pathological processes. They exert their function by catalyzing the hydrolysis of peptide bonds, thereby cleaving proteins into smaller polypeptides and individual amino acids. This proteolytic activity has profound downstream effects, including the modulation of protein−protein interactions, the regulation of protein fate (localization, activity, degradation), the generation of bioactive fragments, and the participation in cellular information processing through the transduction and amplification of molecular signals. Proteases are classified into different types based on its site of action and catalytic mechanism. The classification of proteases is briefly mentioned in Table 6.1.

Tightly regulated proteolytic activity is crucial for maintaining cellular homeostasis. Dysregulation of this balance can significantly impact various physiological processes, contributing to disease pathologies such as uncontrolled cell growth and eventually, cancer. Aberrant protease activity is increasingly recognized as a key contributor to cancer development and progression. These enzymes, crucial for normal cellular proteolysis, turn into oncogenic drivers when their regulation is disrupted. This dysregulation manifests in several ways, promoting tumorigenesis. A prominent mechanism involves the degradation of ECM. Matrix metalloproteases (MMPs) are well characterized proteases that degrade ECM components, facilitating tumor cell invasion and metastasis (Wachtel et al., 1999). Upregulated MMP activity disrupts the physical barrier between tumor cells and surrounding tissues, enabling their migration and infiltration into healthy parenchyma. Beyond ECM

TABLE 6.1 Classification of proteases.

Classification	Description	Example	References
By site of action	**Endopeptidases:** Cleave internal peptide bonds within a protein molecule.	Pepsin, trypsin	Van Der Velden and Hulsmann (1999)
	Exopeptidases: Cleave amino acids from the terminal of a protein molecule.	Carboxypeptidase A, aminopeptidase B	
By catalytic mechanism	**Serine proteases:** Utilize a serine amino acid residue in the active site for catalysis.	Trypsin, chymotrypsin, thrombin	Antalis and Buzza (2016)
	Cysteine proteases: Utilize a cysteine thiol group in the active site for catalysis	Papain, cathepsin B, caspases	Vasiljeva et al. (2007)
	Aspartic proteases: Utilize an aspartate amino acid residue in the active site for catalysis.	Pepsin, renin	Szecsi (1992)
	Threonine proteases: Utilize a threonine hydroxyl group in the active site for catalysis.	Tegumental protease	Boon et al. (2020), Baird et al. (2006)
	Metalloproteases: Require a metal ion (often zinc) as a cofactor for catalytic activity.	Matrix metalloproteinases, ADAM proteases	Chang and Werb (2001)
	Glutamic proteases: Utilize a glutamate amino acid residue in the active site (rare in mammals).	Glutamyl endopeptidase I	Sims et al. (2004)

remodeling, proteases directly influence tumor cell signaling. Cathepsins, a family of lysosomal cysteine proteases, have been implicated in activating oncogenic signaling pathways (Zhang et al., 2016). Their activity can lead to the cleavage and activation of growth factors and signaling molecules, promoting the proliferation and survival of cancer cells. Additionally, proteases modulate the tumor microenvironment by cleaving cytokines and chemokines, influencing the recruitment of protumorigenic immune cells and promoting angiogenesis. Dysregulation of apoptosis, a programmed cell death pathway, is another consequence of aberrant protease activity. Caspases, a family of cysteine proteases, are the key executors of apoptosis. Loss of caspase function or activity can render cancer cells resistant to apoptotic signals, allowing for uncontrolled proliferation (Rahmanian et al., 2016). It is well known that different types of proteases derived either from intestinal epithelium, inflammatory immune cells infiltrated into epithelium or from *H. pylori* contribute to several pathogenesis-associated signaling pathways. Few proteases such as MMPs, serine proteases, and collagenases are known to play a key role in the control and coordination of important events to facilitate

colonization of the bacteria in gastric epithelium and trigger subsequent pathologic events (Sampieri et al., 2013).

4. Role of proteases from *Helicobacter pylori* in GC development

H. pylori infection and subsequent intestinal inflammation are closely linked to the development of GC. This process involves multiple events, which is collectively described as the "Correa cascade" (Correa, 1995, 2013), wherein a progressive transition from chronic gastritis to invasive cancer is observed (Sitarz et al., 2018; Bernegger et al., 2022).

The infection with *H. pylori* activates a complex network of proteases, including MMPs, serine proteases, and collagenases, which contribute to regulation of cellular signaling and ECM destabilization. The role of *H. pylori* proteases, high temperature requirement A (HtrA), Hp1012, and Hp0169 in cleaving ECM molecules and regulation of chemokines, cytokines, as well as their specific cognate receptors to create the pathogenic microenvironment have been reported (Bernegger et al., 2022).

The expression and secretion of the collagenase Hp0169 from *H. pylori* was demonstrated as a prerequisite for bacterial colonization to the host tissue (Kavermann et al., 2003). This protease acts by degrading host type I collagen in the ECM and provides support in bacterial adherence (Kavermann et al., 2003). It is well documented that a number of MMPs are upregulated during *H. pylori*—mediated infection, inflammation, and subsequent gastritis and its transformation into GC (Bernegger et al., 2022). The host protease, MMP-10, has been demonstrated to support *H. pylori* colonization, whereas MMP-7 levels are inversely correlated with bacterial abundance (Krakowiak et al., 2015; Lv et al., 2019a,b). The cytokines, particularly IL-22 released by infiltrating immune cells along with MMP-10, trigger tissue inflammation, which supports in *H. pylori* survival in host stomach. MMP-10 deletion was shown to compromise *H. pylori* colonization in host intestine (Lv et al., 2019a,b).

The chronic tissue inflammation triggered by *H. pylori* infection was linked to Leu171 variant of HtrA and elevated inflammation scores and serum gastric levels (Yeh et al., 2019). Proteolytic impairment of epithelial junction proteins results to loss of barrier functions is known to be strongly linked to the GC pathogenesis. The HtrA released in the host tissue cleaves the transmembrane tight junction proteins, occludin and claudin, thereby impairing the barrier integrity (Sharafutdinov et al., 2022). The cleavage of these tight junction proteins further upregulates MMP-2, MMP-7, and MMP-9, which has been associated with an increase in epithelial or endothelial permeability (Wachtel et al., 1999).

5. Role of host proteases in inflammatory response, cancer cell invasion, and metastasis

Proteases play a critical role in the pathogenesis of GC. Both host-derived and *H. pylori*-associated proteases contribute to epithelial barrier dysfunction, inflammatory processes, and metastatic progression in GC. Proteases are essential mediators of regulation of inflammation, and their enzymatic activity plays a major role in the body defense mechanisms.

They are reported to regulate leukocyte recruitment, coagulation cascade, tissue damage, cytokine, and chemokine activity to initiate and terminate proinflammatory or antiinflammatory responses (Leick et al., 2014; Luiz and Almeida, 2022). Leukocyte activation has been demonstrated to occur during inflammation the possible role of host and pathogen derived proteases in GC development and progression is illustrated in Fig. 6.1.

5.1 Role of serine proteases in GC

Serine proteases are also closely associated with the inflammatory response and tumor progression in GC. They form an essential component of the inflammatory cascade and are released from activated leukocytes (Mugge et al., 1991). Further, the inflammatory response is amplified by activation of other serine proteases involved in kallikrein system, which promotes gastric tissue inflammation (Devani et al., 2005; Stadnicki et al., 1996), release of chymase and tryptase by mast cells (Pejler et al., 2010), and activation of the coagulating cascade, which ultimately results in thrombin formation with locally elevated levels of thrombin activity (Malik et al., 1986; Sharony et al., 2006). Studies performed to evaluate the biological function of a serine protease, PRSS23 in two independent GC cohorts, revealed that its overexpression was clinically correlated with poor prognosis and macrophage infiltration of GC patients (Qin et al., 2022). Another serine protease, TMPRSS4, was also significantly correlated with depth of invasion, lymph node metastasis, clinical stage, and vessel invasion in GC. Based on multivariate analysis, TMPRSS4 expression was identified as an independent prognostic factor and an indicator of poor prognosis in GC patients (Sheng et al., 2014). Also, TMPRSS4 is reported as a key regulator of GC cell

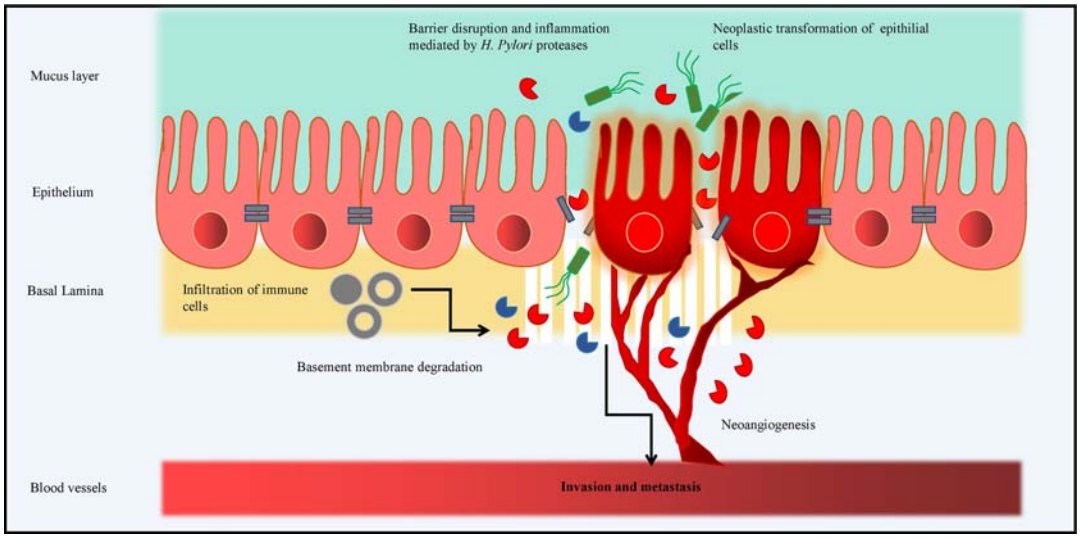

FIGURE 6.1 Role of proteases in GC. Proteases, a broad set of enzymes engaged in protein degradation, are key players in the development and progression of gastric cancer (GC). Proteases from both host and the bacterial pathogen *H. pylori* contribute to intestinal barrier disruption, inflammation, and metastasis in GC.

proliferation, migration, and invasion. Studies performed with TMPRSS4 revealed that its silencing significantly decreases the migration and invasion of GC cells, whereas overexpression of TMPRSS4 significantly promotes cell migration and invasion (Jin et al., 2016). Tryptase released by mast cell secretory granules was correlated with better overall and recurrence-free survival in GC patients (Lin et al., 2017). Another serine protease found in the azurophile granules of polymorphonuclear cells (PMN) or neutrophils is elastase. According to a study reported by Weiss and coworkers, activated PMN produces elastase, which breaks down the ECM components (Epstein and Weiss, 1989). Additionally, it promotes the breakdown of various Interleukins (IL) such as IL-1β, IL-1, IL-8, and IL-2 (Ariel et al., 1998) as well as the release of granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-6, and IL-8 from epithelial cells (Nakamura et al., 1992). Cleavage of both endothelial VE-cadherin and epithelial E-cadherin was linked to increase in permeability through cell layers, which in turn increases leukocyte migration and the spread of inflammation (Carden et al., 1998; Ginzberg et al., 2001). They regulate local immune response by mediating destruction of pathogens caused by their extracellular release upon neutrophil activation at the sites of tissue inflammation (Pham, 2008). Neutrophil elastase is also identified as a potential biomarker for the prognosis of GC and immune cell infiltration in the tumor microenvironment (Jia et al., 2023). Kallikreins (KLK)1−15, a subgroup of serine proteases are released as inactive zymogens that, when activated, can display either chymotrypsin or trypsin-like activity. Inflammation causes them to become overexpressed. They are also implicated in GC progression. Enhanced expression of tissue KLK in tumor and ulcer tissue highlights their role in peptic ulceration and gastric malignancy development (Sawant et al., 2001). Further, KLK6 has been implicated in promoting growth, migration, and invasion of GC cells. Moreover, a significantly high expression of KLK6 in four different GC cell lines (SGC-7901, BGC-823, MKN-28, and MGC-803) was observed as compared with the normal gastric epithelial cells, GES-1. In addition, immunohistochemical analysis revealed stronger staining of KLK6 along with cancer markers vascular endothelial growth factor (VEGF) and MMP-9 in human GC tissue than in normal tissues (Zhu et al., 2018).

5.2 Role of MMPs in GC

The host proteases such as MMP-7 and MMP-10 degrade collagen and also play a key role in *H. pylori* colonization. Upregulation of a number of MMPs is reported in *H. pylori* infection mediated gastritis and GC. Infiltrating B cells and T cells, and cytokines such as interferon gamma (IFN-γ) and IL-17, are reported to be increased in MMP-7 knockout mice indicating its role in gastric inflammation (Ogden et al., 2010). Moreover, MMP-10 was demonstrated to support $CD8^+$ T cell−mediated tissue inflammation, which promotes *H. pylori* survival, and MMP-10 deletion is associated with reduced tissue colonization (Lv et al., 2019a,b). Additionally, expression of several MMPs is enhanced in vivo in *H. pylori* gastritis (MMP-1, MMP-8, MMP-9, and MMP-10) and in tumorigenic tissue (MMP-2, MMP-7, MMP-9, MMP-11, MMP-12, and MMP-14) (Bernegger et al., 2022). The proteolytic composition in the tissue microenvironment affects the inflammatory and chemoattractive properties at the site of inflammation. Further, tissue microenvironment is also noted to impact protease production

(Elkington and Friedland, 2006). Further, it was revealed that IL-21 is massively synthesized in *H. pylori*—infected gastric mucosa, which contributes to Th17 environment and also promotes the upregulated secretion of MMP-2 and MMP-9 (Caruso et al., 2007). Through targeting ECM in the inflamed tissue, proteases such as MMP-9 facilitate the recruitment of immune cell infiltrate, thereby contributing in inflammation and pathogenic tissue remodeling (Bergin et al., 2008). The proteases derived from the epithelium, infiltrating and activated immune cells in combination with *H. pylori* proteases promote GC progression. Apart from having a role in inflammation, MMP-dependent ECM remodeling also directly contributes to gastric ulceration (Kim et al., 2011; Chakraborty et al., 2012).

5.3 Role of cysteine proteases in GC

Cathepsins, a type of lysosomal cysteine proteases that is primarily located inside the lysosomes have been demonstrated to contribute in GC progression and metastasis. An elevation in the expression of cathepsin B decreases laminin, a basement membrane component, facilitating invasion of tumor (Xu et al., 2016). Cathepsin D activity promotes early GC metastasis to lymph nodes, potentially driven by human telomerase reverse transcriptase (hTERT)—mediated activation leading to increased proliferation and invasion (Ikeguchi et al., 2001; He et al., 2016). Additionally, the p53 suppressor anterior gradient 2 (AGR2) upregulates cathepsins B and D, further enhancing cancer cell dissemination (Zhang et al., 2016). Cathepsin K correlates with coronin 3, an F-actin regulator involved in metastasis, suggesting a potential role in promoting metastasis in MKN28-M GC cells (Ren et al., 2012). Forkhead box O3A increases cathepsin L expression, facilitating migration and invasion. Moreover, cathepsin L overexpression suppresses E-cadherin, potentially inducing epithelial-mesenchymal transition and venous invasion (Ren et al., 2012). Cathepsin S shows a positive correlation with GC development and has been proposed as a diagnostic and prognostic biomarker. Its knockdown suppresses cancer cell migration and invasion, and higher serum levels correlate with later stages and poor patient survival according to a study by Liu et al. (2016).

Calpains, a type of nonlysosomal cysteine proteases, are a large group of enzymes within cells that are activated by calcium and play a role in various cellular processes (Goll et al., 2003; Storr et al., 2011). One specific calpain, calpain-4, has potential role in GC development and progression (Suzuki et al., 2004). Elevated calpain-4 mRNA in tumor sample was demonstrated compared with healthy stomach tissue (Stock and Otto, 2005). This suggests that the gene encoding calpain-4 is more active in cancer cells. Secondly, the protein levels of calpain-4 were examined in various GC cell lines alongside a normal stomach cell line (Peng et al., 2016). Most GC cell lines displayed significantly higher calpain-4 protein levels compared with the normal cell line. This finding strengthens the link between calpain-4 and GC by demonstrating an increase in actual protein production within GC cells (Peng et al., 2016).

6. Radiotherapy as a potential treatment modality of GC

RT is an integral part of cancer treatment and uses ionizing radiation in various forms of to minimize the growth, metastasis, and multiplication of malignant tumor cells. It directly

inflicts DNA lesions, including single-strand breaks (SSBs), double-strand breaks (DSBs), inter- and intrastrand cross-links, compromising genomic integrity and hindering crucial cellular processes such as DNA replication and repair (Van der Schans, 1978; Thopan et al., 2017; Dauer et al., 2010). This genotoxic stress can trigger cell death pathways. Furthermore, radiation disrupts the endoplasmic reticulum, activating the unfolded protein response (UPR), ultimately leading to apoptosis if protein homeostasis cannot be restored (Pang et al., 2013; Scheper et al., 2014). Additionally, radiation exposure significantly impacts mitochondria, inducing the release of cyt *c* from the mitochondrial intermembrane space, initiating the intrinsic apoptotic cascade (Kam and Banati, 2013; Fachal et al., 2014).

RT also regulates plasma membrane−related signaling molecules and secondary messengers of neoplasm cells. Radiation also activates mediators that contribute to malignant growth and proliferation. Further, the metastasis and invasive ability of the cancer cells are also enhanced by radiation-induced activation of signaling molecules (Goldkorn et al., 1997; Lammering et al., 2004), which can hamper the cellular metabolic pathway and resulting in apoptosis. Radiation has two effects on cancer cells: it damages DNA to prevent tumor growth, and it also alters the expression of molecules linked to invasion and metastasis to either encourage or prevent the growth of tumors (Park et al., 2006).

There are two types of RT usually employed in GC—intraoperative radiation therapy (IORT) and 3D conformal and intensity-modulated radiation therapy (IMRT). IORT method offers a targeted approach to fight GC during surgery. By shielding healthy tissues, IORT allows delivery of higher radiation doses to the tumor (Hazard et al., 2006).

Technological advancements have led to more sophisticated radiation delivery methods such as 3D conformal radiation therapy (3D-CRT) and IMRT. These techniques offer the potential to minimize side effects on healthy organs while allowing for higher targeted doses to the tumor (Leong et al., 2005). Studies show it delivers lower radiation doses to critical organs compared with traditional methods (Lohr et al., 2003).

Radiation disrupts cellular processes by damaging biomolecules including proteins and enzymes. Ionizing radiation directly interacts with protein molecules, causing peptide bond cleavage, and changes in amino acid side chains (Gabarty et al., 2022). It can also interact with water molecules, generating free radicals that react with protein side chains, leading to their modifications such as oxidation, disulfide bond breakage, and nitrosylation. These changes can disrupt the secondary, tertiary, and quaternary structures of protein further impacting its activity (Kennedy, 2023). Radiation damage can directly disrupt the **active** site of an enzyme, the region responsible for substrate binding and catalysis. This can completely inhibit enzyme activity. Even without complete inactivation, radiation-induced changes can alter the enzyme's conformation, reducing its substrate binding efficiency and performing catalytic reactions (Pałecz and Leyko, 2009)

7. Interplay of proteases and radiation therapy

The established role of dysregulated proteases in GC progression, as previously discussed, necessitates exploration of their complex interplay with RT. While RT is intended to eradicate tumors, its interaction with the proteolytic machinery creates a disordered situation. The intended effect of RT is cause alteration of cellular macromolecules and subsequent apoptosis

in cancer cells. However, some studies suggest that it might inadvertently activate specific proteases, potentially compromising treatment efficacy. RT can directly activate caspases, which are important executioners of apoptosis. This activation can improve radiation efficiency by inducing tumor cell death (Rahmanian et al., 2016). Elevated levels of activated CASP3 in tumor tissue can be a positive indicator of increased radiosensitivity and potential treatment success (Hosokawa et al., 2005). It has been proven that apoptotic receptor agonists, such as monoclonal antibody ligands that bind selectively to TNF-related apoptosis inducing ligand (TRAIL) receptors, in combination with ionizing radiation, generate a synergistic apoptotic impact through the activation of caspase cascade pathway (Marini et al., 2003). Cysteine proteases such as cathepsin B, which is involved in protein turnover and antigen presentation, can be released during RT and show radioresistance (Olson and Joyce, 2015). While this may contribute to tumor cell death, it can also affect nearby healthy tissues, resulting in radiation-induced bystander effect (RIBE). RIBE occurs when factors released by irradiated cells affect adjacent nonirradiated areas, causing genomic instability and altered cell responses (Mothersill and Seymour, 2001). CPR-4, a cysteine protease corresponding to human cathepsin B, was identified as the first RIBE factor in nematodes (Larminie and Johnstone, 1996). CPR-4 activity is controlled by the p53 homolog CEP-1 in response to radiation, and it appears to exert RIBEs via the insulin-like growth factor receptor DAF-2 (Peng et al., 2017).

RT not only kills cells directly, but also causes alterations in the tumor microenvironment that activate MMPs (Waller and Pruschy, 2021). RT has been overlooked for decades, but immunologists are now recognizing its powerful capacity to create a proinflammatory environment conducive to immune cell infiltration. The release of growth factors, chemokines, and cytokines directly affects tumor-stimulating MMP gene expression. At the same time, RT also increases furin gene expression, which results in increased furin-mediated posttranslational conversion of the proform and subsequent activation of many MMPs and all ADAMs/ADAMTS in response to irradiation (Lee et al., 2016; Ra and Parks, 2007). Irradiation produces reactive oxygen species that interact with biomolecules, including MMPs. Cysteine residues in the prodomain interact with Zn^{2+} sites in the catalytic area to regulate MMP zymogens. IR-induced oxidation of key cysteine sites disrupts the inhibitory conformation and activates MMPs (Waller and Pruschy, 2021). Irradiation-induced overexpression of MMP-9 has been linked to increased metastasis and cell invasiveness, as well as influencing treatment outcomes (Chou et al., 2012; Cheng et al., 2006).

8. Proteases as therapeutic targets in GC

Potential therapeutic targets in GC have been identified as the serine proteases and their inhibitors called serpins (Lei et al., 2012). In GC, serine protease modulation is a desirable target for therapy. In a study, it was demonstrated that apoptosis was effectively induced and cancer cell invasion and proliferation were effectively inhibited by blocking the effects of TMPRSS4-mediated cellular signaling. Further, inhibitors of TMPRSS4 serine protease activity such as KRT1853, when administered to nude mice carrying prostate and colon cancer xenografts, effectively inhibited tumor growth. The modulation of TMPRSS4-mediated cellular signaling through inhibitors as IMD-0354 and KRT1853 have the potential to be good anticancer agents (Kim, 2023). As activity of serine protease, TMPRSS4 is reported to increase in many malignant

tumors, it has a significant potential as prognostic biomarker for GC, particularly for stage III. It also correlates with the depth and invasion of the tumor. These roles support it as an effective treatment target for GC (Tazawa et al., 2022). Another case of PRSS23 overexpression in GC patients was clinically linked to poor prognosis and macrophage infiltration. PRSS23 plays an oncogenic role in GC, according to a loss-of-function study indicating that inhibition of this serine protease has a probable ameliorative function in treating GC (Qin et al., 2022).

The MMPs regulate various events of GC development such as extracellular remodeling, angiogenesis, cellular differentiation, proliferation, and apoptosis. They are thought to be crucial targets for cancer treatment because they play a significant role in controlling the growth of tumors both at the main site and in distant metastases (Verma, 2014). Moreover, increased levels of MMPs in tissue, plasma, or serum of GC patients and their positive correlation with tumor invasion, distant metastasis, and tumour, node, metastasis (TNM) stage have considered them as probable diagnostic and prognostic predictors of gastrointestinal cancers. Thus, regulation of MMP activity may be considered as a possible therapeutic target to impede the growth of tumors. Additionally, nucleotide polymorphism (SNP) analysis has the potential to improve illness diagnosis across a range of conditions and predict clinical outcome and therapy efficacy in a number of cancers. Further, inhibition of cysteine proteases such as calpain as supported in preclinical and clinical evaluation has uncovered its ability to reduce carcinogenesis and prevent the spread of aggressive tumors. Clinical evidence indicates that lower survival times are linked to increased expression of calpain-1 and calpain-2 isoforms in cancer. In cell and animal models, genetic abrogation of calpain-1 and calpain-2 is correlated with antitumor effects (Sharafutdinov et al., 2022). Additionally, cathepsin inhibition was observed as a potential target in sensitizing resistant cancer cells to therapy. Furthermore, its ablation was demonstrated to sensitize cancer cells to conventional chemotherapeutic treatments in ovarian and lung cancer. Cathepsins are found to be significantly upregulated in GC; their inhibition was shown to suppress migration and invasion of gastric neoplastic cells and therefore represents a great potential in reducing the spread of GC. Hence, cathepsin may prove a probable biomarker for the diagnosis and prognosis of GC, in addition to being a possible therapeutic target to control disease progression (Soond et al., 2019; Da Costa et al., 2020).

9. Conclusion and future directions

GC presents a significant global health concern, with its development influenced by a complex interplay of several factors. Proteases have multifaceted role in GC pathogenesis, inflammation, tumor progression, and disruption of tight junctions in the gastric epithelium. It is reported that both the host and the bacterial pathogen *H. pylori* contribute to these processes. RT is a well-known treatment option for gastric tumors. Further, the radiation also regulates protease activity resulting in sensitization as well as resistance of GC cells. Understanding this interplay holds immense therapeutic potential in the reduction of GC. By targeting specific proteases or their regulatory pathways, we can potentially develop novel therapeutic strategies to prevent or impede GC progression. Additionally, exploring the impact of RT on protease activity opens new avenues for identification of specific protease targets in different stages of GC development. These targets can then be addressed with selective small

molecule inhibitors or antibody therapies. Developing potent and specific protease inhibitors for clinical use is of paramount importance. These inhibitors could be used alone or in combination with other therapies, such as RT or chemotherapy, to improve treatment efficacy and reduce side effects. Further, understanding the impact of RT on the proteases is crucial. Many studies have highlighted the potential of cysteine proteases, serine proteases, and MMPs as potential therapeutic targets against GC. In conclusion, proteases offer a diverse array of promising targets for GC therapy. Therefore, unraveling the complex interplay between proteases and GC pathogenesis offers exciting possibilities for novel therapeutic interventions (Da Costa et al., 2020). By harnessing this knowledge and pursuing innovative research avenues, we can strive toward a future where GC is effectively diagnosed, treated, and managed.

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CHAPTER

7

Matrix metalloproteases in the pathogenesis of hepatocarcinoma

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Abbreviations

aDAMs A disintegrin and metalloproteinases
ADX Andecaliximab
ECM Extracellular matrix
EGF Epidermal growth factor
EGFR Epidermal growth factor receptor
EMT Epithelial–mesenchymal transition
ER Endoplasmic reticulum
ERK1/2 Extracellular signal–regulated kinase 1/2
GPI Glycosylphosphatidylinositol
HCC Hepatocellular carcinoma (HCC)
HGF Hepatocyte growth factor
IFN-g Interferon-g
IL-1β Interleukin-1β
Mabs Monoclonal antibodies
MAPK Mitogen-activated protein kinase
MMPs Matrix metalloproteinases
MT-MMP Membrane-type MMP
PI-3K Phosphoinositide 3-kinase
PTEN Phosphatase and tensin homolog
SMC Smooth muscle cell
TGF-β Transforming growth factor-β
TIMPs Tissue inhibitors of metalloproteinases
TLR Toll-like receptor
TME Tumor microenvironment
TNF-α Tumor necrosis factor-α
uPA Urokinase-type plasminogen activator
uPAR Urokinase-type plasminogen activator receptor

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VEGF Vascular endothelial growth factor
VEGFR-2 VEGF receptor-2

1. Introduction

Matrix metalloproteinases (MMPs) are xinc-bearing endoproteases, requiring Ca^{2+} or Zn^{2+} ions for activity. MMPs and serine proteases cooperatively degrade ECM proteins composed of collagen, laminin, and fibronectin. Currently, ECM reconstituting MMPs can be grouped to the submembers of gelatinases, collagenases, and stromelysins by their functionality and structure basis. MMPs biosynthesized are subjected to the secretory trafficking and secretion via translational modification of *N*- and *O*-glycosylations in endoplasmic reticulum (ER) and Golgi apparatus. Then, the fate of MMPs is determined as forms of transmembrane or glycosylphosphatidylinositol (GPI)-anchored MT-MMPs, membrane-bound MMPs and secreted MMPs. Among them, the secreted MMP zymogens are activated by extracellular proteolysis to their active enzyme forms. Active MMPs enzymes are controlled by endogenous tissue inhibitors of metalloproteinases (TIMPs) or α2-macroglobulin and also act for ECM degradation (Fig. 7.1). MMPs degrade ECM and are thus called as ECM-reconstituting enzymes in the physiological events. The expression patterns of MMPs are different from tissue locations, and their expression depends on developmental stages because MMPs directly contribute to the ECM turnover during normal cellular process as well as cancer invasion and metastasis. For turnover, MMPs are also controlled by their endogenous inhibitors called TIMPs to maintain balanced ECM catabolism.

They importantly regulate development, differentiation, embryogenesis, embryonic fusion, growth, organogenesis, tissue repair, tissue removal, and wound healing. MMPs tend to be upregulated in tumors and inflamed tissues, as their expressions are upregulated in human diseases. MMPs expression is thus enhanced in cancer cells, cancerous tissues, and inflamed tissues. Simply, MMPs degrade ECM components for the mechanism of cellular migration. Therefore, MMPs participate in differentiation, development, and cellular aging events.

In pathological inflammation process, MMPs regulate wound healing turnover and invasive behavior of cancer (Kim et al., 2023). Since MMPs are considered as valuable candidate targets in the treatment of human diseases, improved clinical studies are waited for the future development.

2. MMPs and biomarkers in liver cancer

Understanding underlying pathological mechanisms is not well elucidated for the development of human hepatocellular carcinoma (HCC) regarded as the leading cause of death worldwide (Gupta et al., 2019). Additionally, relevant approaches to restrict tumor growth and dissemination are limited, and yet to be clearly understood. MMP members are largely implicated in the HCC pathogenesis. This chapter is aimed to present their functions in the HCC development, carcinogenesis, invasion, and progression. Migration and invasion of

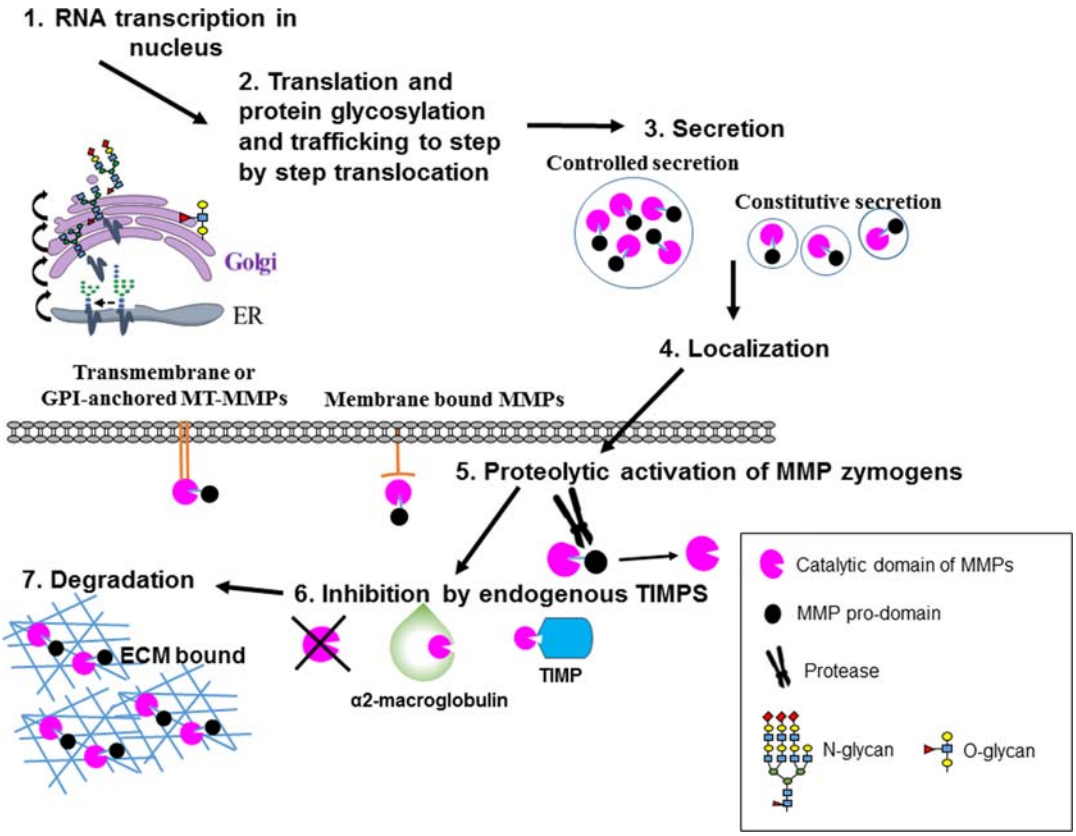

FIGURE 7.1 Biosynthesis, secretory trafficking, and activation of MMPs by extracellular proteolysis. 1. RNA transcription in nucleus. 2. Translation, protein glycosylation, and trafficking lead to step-by-step translocation. 3. Secretion of MMPs from cell. 4. Localization to target positions. 5. Proteolytic activation of MMP zymogens. 6. Inhibition by endogenous TIMPs. 7. Degradation.

liver cancer cells are dependent on the activated MMPs and release to tumor environments. Moreover, their expression on cell membranes is extremely important to catalyze an enzymatic degradation of the ECM components and infiltrate into tissues. The HCC tumor microenvironment (TME) potentiates the invasiveness and metastatic potentials of tumor cells. The interactions of MMPs with TIMPs help to understand their dynamics of cancer cells in liver TME. The limited aggressiveness of HCC is based on the ECM integrity that is determined by normal MMPs and TIMPs ratio. Imbalanced MMP enzyme activity can cross-activate and degrade ECM proteins. The imbalanced MMPs and TIMP inhibitors ratio causes the MMP roles in tumor migration. In other words, TIMP inhibitors balance the MMPs that are activated, and they modulate ECM degradation, angiogenesis, migration, invasion, and progression in HCC. Serum TIMPs and MMP: TIMP ratios stringently correlate with the prognosis of HCC patients and their overall survival. TIMPs are potentially considered to be markers for HCC diagnosis (Daniele et al., 2014).

The carcinoma prognosis depends on the invasiveness via destruction of the ECM in the basement membrane known as a barrier (Scheau et al., 2019). Malignantly metastatic HCC cells express high serum MMP-9 activity. 92-kDa type IV collagenase known as gelatinase B (MMP-9) importantly functions in carcinoma invasive metastasis (Hayasaka et al., 1996). MMP secreted as an inactive proMMP-9 is activated by urokinase-type plasminogen activator (uPA)−generated plasmin (Ramos-DeSimone et al., 1999). MMP-2 gene is constitutively expressed in various cell types, but MMP-9 expression is elevated by metastatic progression of cancer cells (Chung et al., 2004a,b). Inactive proMMP-9 requires activation by plasmin and uPA plasmin (Ramos-DeSimone et al., 1999; Chung et al., 2002) have found that the activation of proMMP-9 was mediated by plasmin (Fig. 7.2).

2.1 Mechanistic expression of MMP-9

It is modulated through ERK/AKT/PKB/PI-3K/AP-1/NF-κB signaling pathway. The promoter region of MMP-9 transcription has the consensus sequence in AP-1 and NF-κB-binding sites. Chung et al. (2004a,b) identified the AP-1 and NF-κB-binding sequences

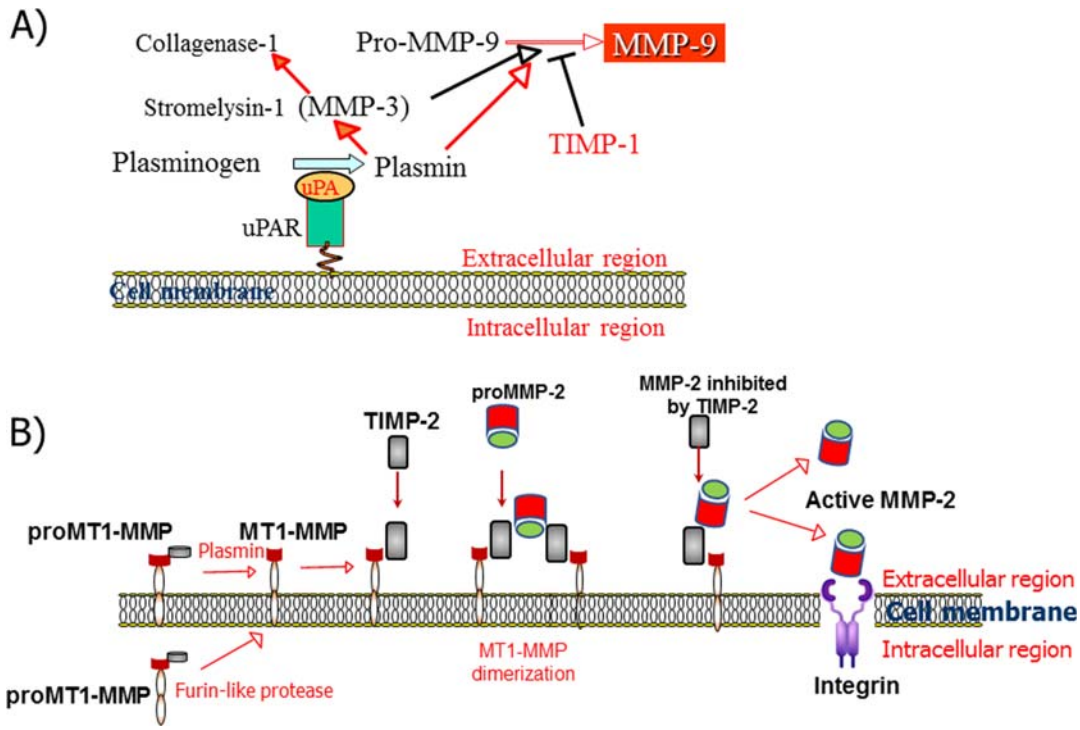

FIGURE 7.2 MMP-9 activation cascade at cell surfaces. The MMPs activation coordinates and is started by the plasmin formation. Urokinase-type plasminogen activator (uPA) is attached with uPA receptor (uPAR), and plasmin activates stromelysin-1 and MMP-9 activities. Stromelysin-1 activates MMP-9 and collagenase-1(A). MMP-2 activation is carried out by cell-surfaced MT-MMPs (B).

from MMP-9 transcriptional promoter sequence in hepatitis B viral x gene-transfected cells. MMP-9 promoter region that carries the AP-1 and NF-κB cis-elements induces MMP-9 gene expression using 710 bp region that contains the TATA box, AP-1, and NF-κB-binding sites. ERKs activation is occupied by transactivation and AP-1 and NF-κB DNA binding required for the transcriptional promoter region of MMP-9 gene.

MMP-9 activity increase is balanced with decreased TIMP-1 and TIMP-3 activities in metastatic liver cancer cells. MMPs activities are modulated by endogenous TIMP inhibitors forming their complexes through 1:1 stoichiometric interaction with MMPs. The shortage of TIMPs quantity of TIMP-1 and TIMP-3 reflects the elevated MMP-9 activity in metastatic HCC cells. TIMPs also have growth factor—like activity. Among the TIMP family, TIMP-1 recognizes MMP-9 protein to bind and inhibit the MMP-9 activity. The additionally remained MMP-9 activity is allowed by the low TIMP-1 and TIMP-3 expression levels in metastatic HCC cells. The expression levels of TIMP-1 and TIMP-3 are strictly inhibited in metastatic HCC cells. Thus, lowered TIMP-1 and TIMP-3 activities reflect the suppressed TIMP-1 and TIMP-3 expressions in invasive HCC cells. The expression levels of TIMP-1 and TIMP-are scarcely present in invasive HCC cells, whereas TIMP-2 level is significant. However, TIMP-1, -2, and -3 are highly present in normal liver cells or nonmetastatic HCC cells. Thus, the expressed MMP-9 level is correlated with the suppressed levels of TIMP-1 and TIMP-3 in invasive and metastatic HCC cells. Hepatitis B virus (HBV)—infected HCC malignancy exhibits MMP-9 expression but TIMP-1 and TIMP-3 suppression. Similarly, HCC malignancy holds MMP-9 activity but not MMP-2 activity, despite TIMP-2 presence. Therefore, TIMP-1 and TIMP-3 are known to be absent in malignant tumor cells. TIMP-1 is specific to both inactive and active forms of MMP-9, but TIMP-2 to inactive and active forms of MMP-2. The malignant cells do not express TIMP-1 in HCC. Although TIMP-1 and TIMP-2 inhibit vascular tube formation endorsed by endothelial cells (ECs) and angiogenesis in vivo (Chung et al., 2004; Kim et al., 2023), TIMPs also potentiate development of HCC (Chung et al., 2014).

Collagenase-1 known as an MMP-1 type degrades the interstitially synthesized collagen forms and TIMP-1 regulates MMP-1 activity in HCC. In HCC cells, MMP-1 and TIMP-1 expression is tightly linked to regulate the enhanced invasion and migration through enzymatic ECM breakdown (Scheau et al., 2019). Although MMP-2 (or collagenase) as the major enzyme type of MMPs is not expressed in normal hepatocytic cells in liver, the expression of MMP-2 enzyme is upregulated in fibrolamellar variant cells and HCC cells. MMP-2 expression is upregulated by hypoxic HIF-1α known as a tumor invasion and metastasis inducer to enhance MMP-2 transcription (Jing et al., 2012). MMP-3 (or stromelysin-1) digests ECM substrates.MMP-3 expression is stimulated by hepatocyte growth factor (HGF) to invade ECM in liver cancer cells. MMP-3 enzyme is pivotally associated with the HCC invasion (Monvoisin et al., 2002). Except for HGF, other cytokines of interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and interferon-g (IFN-g) also stimulate the HCC invasion and migration via transcriptional activation of MMP-3 gene (Okamoto et al., 2010). MMP-7 matrilysin degrades the ECM. It cleaves both precursors, pro-MMP-2 and pro-MMP-9, to mature forms (Yokoyama et al., 2008), serving as a prometastatic factor in HCC. MMP-8 (or collagenase-2) enhances cell proliferation, migration, and angiogenesis (Fang et al., 2013). MMP-8 stimulates the PI3K/Akt/Rac1 axis signaling in HCC to elevate invasion and migration levels (Qin et al., 2016).

Together with MMP-2, MMP-9 has been mostly studied in liver cancer cells such as HCC. MMP-9 enzyme digests the ECM substances and cleaves chemokines and cytokines such as IL-1β to elevate their functional activities. MMP-9 is directly associated with tumor angiogenesis through its intervention of angiogenesis and endothelial cell recruitment (Heissig et al., 2002). MMP-9 in HCC helps to invade into lymph node region and also promotes metastasis with poor prognosis (Sun et al., 2018). MMP-9 is, therefore, an invasive marker related to metastasis.

Stromelysin-2 known as MMP-10 is mainly found in epithelial cells and regulates cancer cell invasion and metastasis via cleavage of other pro-MMPs and subsequently degrades the ECM components (Zhang et al., 2014). MMP-10 develops HCC, angiogenesis, and growth. CXCR4, stromal-derived factor-1, and c-Jun upregulate MMP-10 expression in HCC cells (Gao et al., 2018). MMP-11 (or stromelysin-3) shows its limited substrate usage for its catalysis. As MMP-10 substrates, insulin-like growth factor—binding protein-1 (IGFBP), laminin receptor (LR), and native collagen VIa3 chain are regarded as poor prognosis factors in HCC. IGFBP and LR are specifically cleaved by MMP-10. MMP-12 macrophage elastase degrades elastin. MMP-12 affects overall survival rate of HCC patients but is not related to HCC invasiveness or metastasis (Sun et al., 2018). In HCC invasion and metastasis, MMP-13 (collagenase-3) degrades the basement membrane and ECM substances, while transforming growth factor-β (TGF-β) upregulates MMP-13 expression level (Sume et al., 2010). MMP-14 that is classified as a membrane-type (MT) degrades the ECM and involves in cancer metastasis. MMP-14 activity enhances the secretion potentials of both pro-MMP-2 and pro-MMP-9 by interaction with TIMP-2 inhibitor. MMP-14 expression in HCC is correlated with its recurrence, portal vein invasion, and intrahepatic metastasis (Li et al., 2014). MMP-14 expression inhibitor, pravastatin, decreases HCC invasiveness and metastatic potentials by decreased MMP-2 activation (Taras et al., 2007). MMP-15 as the MT-MMPs acts for pericellular proteolysis and involves in human fibrosarcoma, gastric cancer cell growth, tumor progression, and intratumoral angiogenesis. However, MMP-15 involvement in HCC is not elucidated yet (Sun et al., 2018).

MMP-16 as an MT-MMP type located on the fibroblast surfaces degrades ECM proteins and activates pro-MMP-2 to active MMP-2 (Jung et al., 2009). MMP-17 as an MT-MMP is a GPI-anchored protein. MMP-17 is associated with epidermal growth factor receptor (EGFR) signaling-driven breast cancer progression not by a protease action (Wang et al., 2017). MMP-19 (stromelysin-4) regulates neovascularization, angiogenesis, and lymphocyte extravasation. MMP-20 (enamelysin) as a tooth-based MMP in present in cancer cells such as breast, colon, and lung cancers. MMP-20 degrades ECM to help tumor cell invasion and migration. MMP-21 cleaves the embryonic node targets and activates latent TGF-β. MMP-21 activity potentiates the cell adhesion and migration of HCC that are mediated by the catalytic vitronectin-like domain in HCC. MMP-23 lacks C-terminal transmembrane domain of GPI anchor. MMP-23 expression is increased in a chronic inflammatory HCC in the hepatocarcinogenesis. MMP-24 activates MMP-2 and is a marker of lung and gastric adenocarcinoma invasiveness. MMP-25 as GPI-anchored MT-MMP of human cancers is found in urothelial, brain, colon, and prostate cancers. Matrilysin-2 (named MMP-26) degrades ECM components and promotes pro-MMP-9 to MMP-9 form. FGF enhances HCC proliferation and migration by MMP-26 activation in HCC Huh-7 cells (Wang et al., 2017). MMP-27 is located by retention in the ER and a poor prognosis marker in cancer patients such as breast

tumor and HCC, as it elevates HCC invasiveness. MMP-28 (or epilysin) degrades casein and is related with a poor HCC prognosis.

3. Therapeutic MMP inhibitors in human diseases

The MMPs can importantly prevent and treat various MMP-dependent diseases. The MMP-mediated pathogenic diseases are mainly related to the inflammatory cell proliferation in human tissues. This indicates that they can be used as potentially diagnosis or treatment for the diseases. MMPs regulate cancer and inflammation but are also required for normal physiology. Thus, reconsideration for an MMP inhibition strategy with the broad spectrum has been considered. Although MMPs have been regarded as an intriguing target, MMP inhibitors have not been applied for clinical treatments due to their unexpected adverse effects. MMP expressions are associated with the invasive and metastatic potentials of inflamed tissues and cancer cells; therefore, MMP inhibitors and functional roles of MMPs have been targeted. Progression of inflamed tissues such as cancer correlates with the ECM. MMPs activation and inhibition interested in their function are attractive for their physiological functions in body homeostasis. Various types of MMP inhibitors have been developed, but clinical trials for these MMP inhibitors have conveyed their side effects. For example, MMP-9 inhibitors have been useful in metastasis inhibition. For prevention of tumor metastasis, some MMP inhibitors are currently under investigation. Since MMP inhibitors with broad spectrum suggested the possible side effects on arthralgia, myalgia, stiffness, and edema. Natural products such as organosulfur compounds, limonene, resveratrol, and polyphenols show MMP inhibitory activities. For orally active agent, natural products have been known to have anticancer activities. They include limonene, resveratrol, green tea polyphenols, and organosulfur compounds.

Polyphenol MMP-9 inhibitors of quercetin, caffeic acid (CA), CA ester, 3,4-dihydroxyphenyllactic acid, CA phenethyl ester (CAPE), chlorogenic acid (caffeoylquinic acid), 5′-caffeoylquinic acid, rosmarinic acid (ROS), epicatechin-3-gallate (ECG), epigallocatechin (EGC), and EGC-3-gallate (EGCG) have been known (Moon et al., 2003). For example, flavonoid quercetin in the human diet protects the vascular lesion, and quercetin's antiatherogenic and biological effects are exerted through MMP-9 downregulation and plaque rupture prevention. Quercetin's repressive effect is based on the decreased transcription level of the MMP-9 gene via blocking NF-κB and AP-1-binding capacities to repress TNF-α-exerted expression of MMP-9 gene. For EGCG, tea polyphenols such as catechins of EGC, EGCG, and ECG. EGCG suppresses G_1 to S-phase progression in cell cycle and AP-1 and NF-κB-drived MMP-9 gene expression in transcriptional level of TNF-α-induced vascular SMCs. EGCG's MMP-9 inhibition is featured by the decreased expression and enzyme activity stimulated by TNF-α by the decreased AP-1 and NF-κB-binding activity to DNA (Moon et al., 2003). Especially, EGCG as plant polyphenol strongly inhibits the gelatinolytic MMP-2 and -9 activities as well as the elastinolytic MMP-12 activity (Demeule et al., 2000). CA and its derivative CAPE (Cha et al., 2003; Jin et al., 2005) show their selective MMP-2 and -9-inhibitory activities, but do not inhibit MMP-1, -3, -7, or cathepsin K activity. The oral administration and subcutaneous treatment of CA and CAPE also effectively inhibit the metastatic capacity

in liver tissue. CA and CAPE suppress the 5′-flanking region activity of MMP-9 gene promoter with the reduced MMP-9 mRNA level. CA and CAPE suppress the phorbol myristate acetate (PMA)−induced NF-κB activation, but not PMA-induced AP-1 activation.

As a new MMP-9-inhibitor, a phenolic ROS-A could be used for the cancer chemotherapy and EGCG also inhibits MMP-9 activity. ROS-A inhibited the invasion dose-dependently. Carboxymethyl ascochlorin, esculentoside, pimaric acid, *Cliona celata*, and *Oldenlandia diffusa* extracts also inhibit expression of inflammation cytokines and MMP-9 via AP-1/NF-κB/mitogen-activated protein kinase (MAPK)/Toll-like receptor-4 (TLR-4) axis in lipopolysaccharide-treated RAW264.7 cells and human aortic SMC migration (Ha et al., 2019; Park et al., 2019; Suh et al., 2012; Chung et al., 2017). However, side effects such as skeletal pain and inflammation are occurred in clinical trials with metastatic cancer patients by objective drugs that inhibit the MMPs catalytic active site. Thus, the therapeutic use of MMP inhibitors is subjected to reexamination. Instead, several selectively acting inhibitors are developed to treat acute and chronic inflammation diseases toward expectation to the future development. Since 2002, MMP inhibitors targeted for different MMPs domains showed positive parameters in treating inflammation tissues and cancers without side effects during clinical trials (Fields, 2019).

Selective dual activities with MMP-2 and -9 inhibitions are promising as anticancer drugs. Several synthetic inhibitors of MMPs are under current clinical trials for cancers. The clinical challenges of MMP inhibitors to treat MMP-related diseases are caused by the uncertain MMP-dependent disease etiology.

Monoclonal antibodies (Mabs) against MMP-9 or MMP-14 were evaluated in breast tumors, gastric/gastroesophageal junction adenocarcinoma, and ulcerative colitis (Shah et al., 2018). In contrast to compounds, monoclonal antibodies are more specific to target MMPs with fewer side effects and toxicity. Demerits of Mabs are their limitation in capacity to enter into cancer cells and production costs. Therapeutic Mab named D1(A12).45 D1(A12) generated against ADAM17 recognizes both the catalytic domain and disintegrin/Cys-rich domain of ADAM17 (Tape et al., 2011). D1(A12) prevents integrins attachment to the disintegrin/Cys-rich domain of ADAM17. Another Mab MED13622 against ADAM17 inhibits cancer cell growth in preclinical trials. Andecaliximab (ADX) as a MMP9 inhibitor is effective in clinical trials of pancreatic and gastric adenocarcinoma.

Commercial MMP inhibitors are available from market. Marimastats are historically known as products GI-5712, BB-2516, or KB-R-8898 and broadly inhibit MMPs in its substrate spectrum. Marimastat is a promising agent useable for a preclinical test and acquired phase II and phase III trials for tumors. As nonpeptidomimetic MMP inhibitor, tanomastat known as BAY 12-9566 inhibits MMP-2, -3, and -9. Nonpeptide inhibitor prinomastat known as AG3340 or KB-R-9896 inhibits MMP-2, -3, -7, -9, and -13. Metastat, named COL-3, CMT-3, or incyclinide, inhibits MMP-1, -2, -8, -9, and -13. As an orally active MMP inhibitor, CMT-3 exhibits multiple proteases inhibitory activities, and this is under development by CollaGenex via phase II trials for the therapeutic metastatic cancers and Kaposi's sarcomas. BMS-275291 has been developed as a broad-spectrum MMP inhibitory agent. Naturally occurring multifunctional antiangiogenic drug neovastat, called AE-941, inhibits MMP-1, -2, -7, -9, and -13, as is under phase III clinical trials. Although the test outcomes obtained from the MMP inhibitor neovastat are not satisfactory due to the side effects, the development of MMPs-specific inhibitors is hopeful in future. Low-molecular-weight MMP inhibitors based on hydroxamate

also inhibits the ADAM10 and ADAM17 as dual inhibitors for anti-cancer capacity (Mullooly et al., 2016). INCB3619 of methyl (6S,7S)-7-(hydroxycarbamoyl)-6-(4-phenyl-3,6-dihydro-2H-pyridine-1-carbonyl)-5-azaspiro[2.5]octane-5-carboxylic acid structure has been developed by Incyte. INCB3619 also inhibits MMP-2 and MMP-12 (Zhou et al., 2006). INCB3619 does not cause musculoskeletal side effects in the model animals. INCB7839 and INCB3619 are structurally related inhibitory agents. INCB7839 has a distinct structure of (6S,7S)-7-[(hydroxyamino)carbonyl]-6-[(4-phenyl-1-piperazinyl)carbonyl]-5-azaspiro[2.5]octane-5-carboxylic acid methyl ester was developed by Incyte (Newton et al., 2010). However, clinical trials in breast cancers with INCB7839 are not carried out. INCB7839 currently undergoes a phase I/II clinical trial to regress non-Hodgkin's lymphoma, diffuse large B cells (NCT02141451) patients. PF-5480090 (WAY-18022 or TMI-002) developed is a selective ADAM17 inhibitor of Pfizer and also is an MMP8 and MMP13 inhibitor.

Selective and multitargeting MMP inhibitors showing superior inhibitory activity at the picomolar potency represent future disease therapies. Currently, most known MMP-inhibitory agents are known against MMP-9- or MMP-13. Recent proteolysis-targeted chimeras (PROTAC) approaches greatly improved the selectivity of MMP inhibitors. MMP inhibitors have now been entered to clinical trials and are hopeful to develop to meet the desired pharmacology by combination chemistry.

Potent strategic approaches for reducing the level of MMPs in arterial tissue have been illustrated in Fig. 7.3. *N*-isobutyl-*N*-4-methoxyphenylsulfonyl glycyl hydroxamate as a well-known MMP inhibitor shows a broad-inhibitory spectrum to MMP enzymes, as it inhibits the invasiveness of the cells (Woo et al., 2019). From plants, dehydrocostus lactone purified from *Aucklandia lappa* inhibits the MMP2 and MMP9 expression in macrophages and suppressed the macrophage polarization (Stejskalová et al., 2021). An antiinflammatory natural product, resveratrol also inhibits MMP activity. In case of combination of synthetic drugs, synthetic drug cisplatin combined with letrozole inhibits MMP2 activity, new blood vessel formation, and angiogenesis.

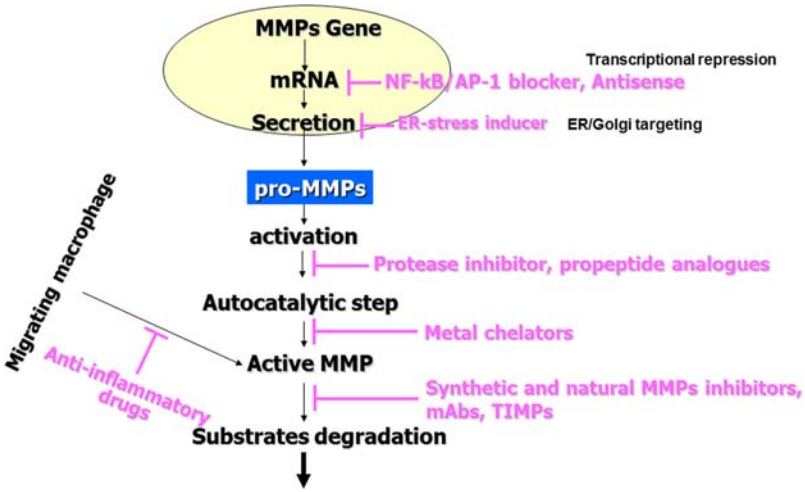

FIGURE 7.3 Strategy of MMPs-related therapeutic drugs in inflammatory cells and liver cancer cells. Potent strategic approaches also reduce the level of MMPs in innate immune cells, cancer cell, and arterial intimal tissues.

4. Conclusion in human diseases

The MMPs are suggested as progression indicator of the disease (Raeeszadeh-Sarmazdeh et al., 2020). Angiogenesis is also initiated by MMPs. Epithelial characteristic-defective cells try to acquire the mesenchymal cell-phenotyping features, calling epithelial–mesenchymal transition (EMT). The EMT process loses polarity, cell adhesion, and migration capacity. This state increases in the migration and invasion capacity because the state is beneficial for the new lesion formed by EMT. In liver hepatoma, the MMPs activation coordinate to form active plasmin by uPA/uPAR, and plasmin activates MMP-9 and stromelysin-1. Stromelysin-1 and MT-MMPs activate MMP-9, MMP-2, and collagenase-1 in development and metastatic invasiveness of liver hepatoma. Gelatinase MMP-2 and -9 are targets for therapeutic strategy in tumors. Anti-MMP inhibitory substances and compounds have been developed for human patients. Currently, various MMP inhibitor candidates are under clinical use and clinical trials for MMP-1, -2, -3, -7, and -9 subtypes. For example, the targets of such MMP inhibitors are mainly focused on anticancer therapies of several cancers including liver, breast, gastric, ovarian, prostate, and renal cancers. The resources of the current MMP inhibitors are based on nonpeptide compounds, synthetic peptides, tetracycline analogs, bisphosphonates, or natural TIMP-1 species.

Conflict of interest

The author declares that this chapter has been written in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Author contributions

C–H K wrote this chapter and read and agreed the final version of this chapter.

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CHAPTER

8

Exploring synthetic and natural compounds for cathepsin B: Mechanisms and therapy

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1. Introduction

Breast cancer (BC) is a heterogenous type with complex etiology, portrayed with abnormal growth and uncontrolled proliferation of atypical cells that invade adjacent tissues as the tumor progresses but disseminates to distant organs at advanced stage (Liang et al., 2020). Despite significant advances in diagnosis and treatment, BC remains a leading cause of death globally (Fan et al., 2020). Proteases are a class of enzymes that critically function in cancer progression by facilitating drug resistance and metastasis via degrading extracellular matrix (ECM) proteins and inducing tumor angiogenesis (Vasiljeva et al., 2021). Cathepsin B (CatB), a cysteine protease, has been found in a variety of normal tissues as well as cells, where it is implicated in the degradation of proteins and turnover of organelles (Fujii et al., 2014). Further, the abnormal expression is implicated in cancer growth, invasion, metastasis, and drug resistance (Saha et al., 2021). In BC, catB was secreted into the extracellular space, where it cleaves ECM proteins, a critical step in progression of primary breast tumors to the metastatic (Kilar et al., 2020). It also supports the establishment of a premetastatic niche, which is a specialized microenvironment that facilitates the growth as well as the survival of disseminated tumor cells. The niche is formed by the recruitment of myeloid-derived suppressor cells (MDSCs) as well as tumor-associated macrophages (TAMs), which secrete factors that promote tumor angiogenesis (Lin et al., 2019).

Recent studies have also implicated catB in drug resistance in cancer. CatB expression is upregulated in drug-resistant cancer cells (Mijanović et al., 2019). In addition, CatB has been shown to mediate resistance to different classes of chemotherapeutic agents, including taxanes, anthracyclines, platinum compounds, and tyrosine kinase inhibitors. The

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mechanisms by which CatB contributes to drug resistance are multifactorial and complex. CatB can directly degrade chemotherapeutic agents, preventing their accumulation and cytotoxicity. It can also modulate the expression of drug transporters, such as P-glycoprotein (P-gp), which effluxes drugs from cancer cells. Furthermore, CatB can promote the survival and proliferation of drug-resistant cancer cells by activating prosurvival signaling pathways, such as the Akt and NF-κB pathways (Zhong et al., 2013).

CatB is also reported to involve in the immune evasion of cancer by cleaving immune-related proteins and generating immunosuppressive peptides (Jakoš et al., 2019). It can cleave MHC class II molecules responsible for presenting antigens to T cells, leading to impaired antigen presentation and T cell activation. It can also cleave the L-selectin on T cells, which is involved in T cell trafficking to secondary lymphoid organs, leading to impaired T cell migration and tumor infiltration. Furthermore, CatB can cleave cytokines, such as IL-2 and IFN-γ, and generate immunosuppressive peptides that inhibit T cell proliferation and activation. In addition, CatB can also promote the recruitment of MDSCs, which are known to inhibit T cell function and promote tumor progression (Chen et al., 2019). Additionally, CatB can cleave the chemokine CXCL12, which recruits MDSCs and increases MDSC infiltration into tumors. A recent finding has shown that CatB regulates the immune checkpoint protein PD-L1. CatB can cleave PD-L1 and generate a soluble form that can bind to PD-1 on T cells, leading to T cell exhaustion and reduced T cell function (Senjor et al., 2023).

Several natural compounds have been identified as potential inhibitors of CatB (Akinyemi et al., 2021; Vidal-Albalat and González, 2016). They can directly or indirectly inhibit CatB activity by affecting its expression or localization. Also, synthetic compounds are reported to inhibit CatB in cancer cells (Saroha et al., 2022). Natural compounds and their derivatives are attractive candidates for cancer therapy because of their low toxicity and diverse chemical structures (Atanasov et al., 2021). Therefore, synthetic and natural compounds that inhibit CatB activity or expression in cancer are an active area of research, and considerable progress has been made in recent years. A complete study of the mechanisms of action of these compounds on CatB and their impact on BC signaling pathways is critical to developing effective therapies for treating drug-resistant BCs.

This chapter provides an overview of the impact of synthetic and natural compounds that target CatB, their mechanism of action in BC, and their therapy response. The first part of the chapter covers the current understanding of the molecular mechanisms underlying CatB-mediated cancer progression, metastasis, and drug resistance. This includes the effects of CatB on ECM remodeling, angiogenesis, immune evasion, and drug metabolism. The second part of the chapter focuses on the potential of CatB inhibitors to enhance the efficacy of chemotherapy. The final part of the chapter provides insights into the state of CatB-targeted therapies and their potential as a novel approach to cancer treatment.

2. Metastasis of breast cancer

Metastasis is a severe complication that can arise in BC patients due to immune evasion and failure of therapy. It is a pivotal contributor to morbidity as well as mortality (Mendez and DeAngelis, 2018). Understanding the molecular mechanisms essential for the progression

of BC to metastasis is crucial for developing effective therapeutic strategies that can improve patient outcomes. Histochemical analysis of 188 BCE tissue specimens showed that CatB expression is associated with ER expression. High levels of CatB expression were also linked with poor disease-free survival in BC patients. Univariate analysis indicated that both the expression of CatB protein and BC metastasis to the bone were associated with poor disease-free survival, highlighting the potential of CatB as a prognostic factor in BC patients (Sun et al., 2016).

2.1 Cathepsin B mediates breast cancer development and progression

The protease CatB contributes to the transformation of breast epithelial cells (Aggarwal and Sloane, 2014), leading to the premalignant progression of these cells by facilitating the degradation of ECM, including collagen and fibronectin. The breakdown of ECM can create space for the expansion of the TME, which can ultimately contribute to tumor growth and progression (Mullins et al., 2012). Sameni et al. found that the peripheral surface of ras-transfected MCF10AneoT cells was stained with CatB through immunofluorescence. This staining pattern was also observed in MCF-7 and BT-20 cells. The study provided evidence that the dysfunctional trafficking of CatB contributes to the transformation of breast epithelial cells to malignant cells. Additionally, it was observed that vesicles containing CatB relocated to the cell surface during the transformation of preneoplastic to neoplastic state, and this process persisted as a characteristic of the malignant phenotype in BC cells (Sameni et al., 1995).

CatB is known to initiate proteolytic pathways crucial for IBC invasion. In a clinical study, 23 patients with IBC and 27 patients without IBC were enrolled, and untreated patient tissues were used to analyze the levels of CatB and caveolin-1 (cav-1) using immunohistochemistry and immunoblotting. In both groups, the study discovered a correlation between the CatB and cav-1 expression as well as the number of lymph nodes positive for metastasis. The results propose that CatB could be a valuable prognostic indicator for predicting the metastasis of tumor cells to lymph nodes in patients diagnosed with IBC (Nouh et al., 2011). Another study explored the role of CatB in spontaneous mammary metastasis to the bone by using stefin A, a cysteine cathepsin inhibitor, in a clinically relevant 4T1-derived syngeneic murine model. The study found that in primary tumors, the expression of stefin A was inversely related to metastatic potential and was induced within the TME.

Moreover, the overexpression of stefin A in the highly metastatic 4T1.2 cells led to significant suppression of CatB expression, which resulted in the inhibition of spontaneous bone metastasis after orthotopic implantation of tumor cells into the mammary pad. The results agreed with the mouse data and furnished proof that CatB facilitates distant metastasis in a group of primary tumors obtained from BC patients (Parker et al., 2008). Another study has reported that CatB overexpression is associated with carcinogenesis through the ErbB pathways. E-cadherin, a substrate for CatB, plays a crucial role in adherens junctions, and its suppression in cancer has been directly correlated with metastasis. Recent research suggests that CatB also plays a role in TAMs in the TME (Fig. 8.1). In addition, CatB is required for lysosomal membrane permeabilization, a cellular process, and prevents chemotherapeutic agent−induced cell death in cancer cells (Ruan et al., 2015).

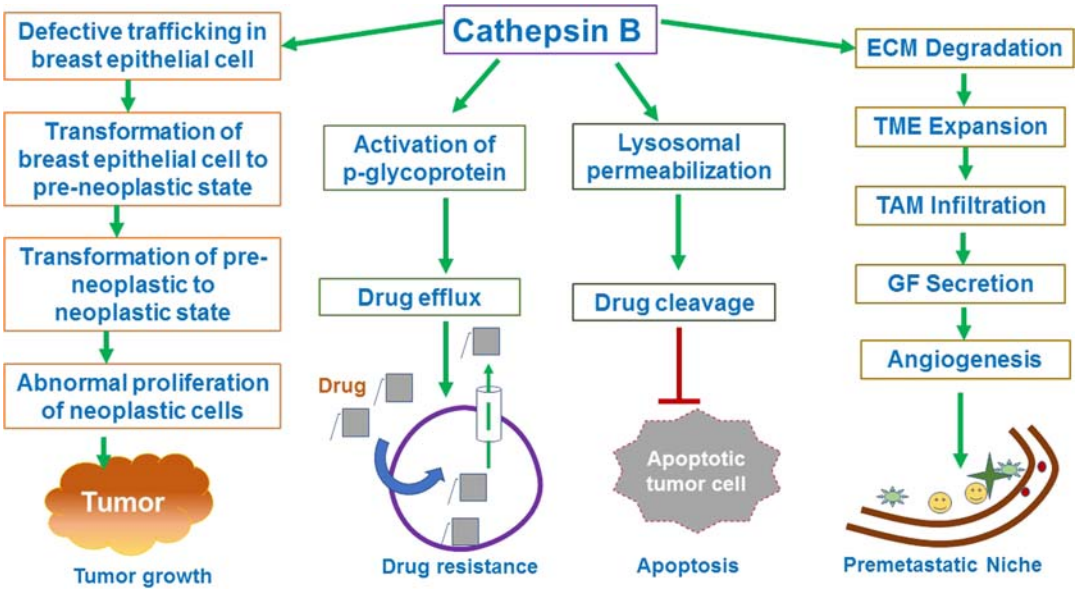

FIGURE 8.1 The role of cathepsin B in breast cancer development and progression. Defective trafficking of CatB in breast epithelial cells leads to its transformation into premalignant and subsequently neoplastic cells, which abnormally proliferate into malignant tumors. CatB activates p-glycoprotein causing drug resistance by effluxing drugs. Additionally, lysosomal permeabilization of CatB results in the cleavage of drug molecules, leading to evasion of drug-induced apoptosis. CatB facilitates ECM degradation, leading to the expansion of the tumor microenvironment by infiltration of TAMs. These TAMs secrete growth factors leading to the induction of angiogenesis and subsequently the formation of a premetastatic niche.

2.2 Regulation of cathepsin B in breast cancer

Studies in the past decade have reported that CatB is regulated by cytokines, chemokines, cochaperones, and cell surface vacuolar-type—ATPase (V-type ATPase) in BC (Fig. 8.2). A study by Ibrahim et al. discovered that abnormal levels of interleukin-6 (IL-6) and the CatB are linked to a poor prognosis in BC. Additionally, treatment with recombinant human IL-6 in MCF-7 cells led to increased invasiveness potential, along with elevated CatB, which was associated with a decrease in E-cadherin and an increase in vimentin protein expression. Notably, a positive correlation was reported between IL-6 and CatB in clinical samples of ER-positive BC patients with axillary metastatic lymph nodes. These findings suggest that CatB, dependent on IL-6, is essential in tumor cells to lymph node (Ibrahim et al., 2016). In a separate investigation, the hormone receptor-positive MCF-7 cells and hormone receptor-negative MDA-MB-231 cells were reported to express both CXCR3 splice variants A and B, as well as CatB. This study reports that CXCR3 ligands can modulate the expression of CatB. Specifically, binding of CXCR3-A-specific C-X-C motif chemokine ligands CXCL9 and CXCL10 to their corresponding receptors induces upregulation of CatB in BC cells, indicating a potential regulation of CatB by chemokines (Bronger et al., 2017). In the TME of IBC, TAMs secrete cytokines such as IL-8 and MCP-1 at high levels. They have been found to induce the expression of CatB as well as the activation of the p-Src/p-Erk1/2 signaling, which promotes

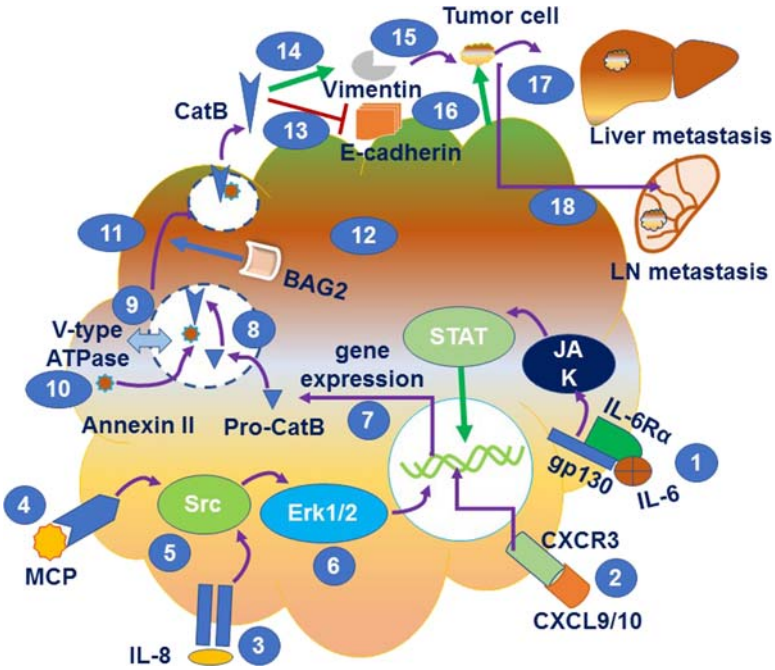

FIGURE 8.2 Regulation and function of CatB in breast cancer. (1) IL-6 induces pro-CatB gene expression by activating JAK/STAT signaling via binding to IL-6R. (2) Chemokines CXCL9/10 induce the expression of the pro-CatB gene through CXCR3-dependent signaling. (3–6) IL-8 and MCP activate Src/Erk1/2 signaling by binding to their specific receptors. (7) These signaling pathways transcriptionally induce the expression of pro-CatB. (8) Pro-CatB is imported into vehicles. (9) Pro-CatB is activated to CatB by V-type ATPase through mannose-6-phosphate receptor-dependent trafficking. (10) CatB forms a complex with annexin II. (11–13) BAG2 facilitates the transport of vesicles near the periphery of tumor cells and their secretion. (14–16) In the extracellular region, CatB reduces E-cadherin and enhances vimentin protein, facilitating the detachment of tumor cells. (17) Tumor cells disseminate to the liver through the bloodstream. (18) Tumor cells disseminate to lymph nodes.

metastasis. Additionally, the downregulation of CatB expression by dasatinib as well as U0126 was observed. The inhibitory effect on CatB activity in triple-negative IBC was found to be counteracted by the presence of IL-8 and MCP-1/CCL2, highlighting the regulatory function of these two molecules on CatB (Mohamed et al., 2020).

CatB is also reported to be regulated by cochaperone Bcl-2-associated athanogene 2 (BAG2), which has been shown to overexpress in TNBC significantly. When BAG2 is silenced, it reduces tumor growth as well as metastasis of BC cells to the lungs. It further induces apoptotic cell death by enhancing the maturation of intracellular CatB. Conversely, BAG2 overexpression triggers metastasis via interacting with the propeptide region and blocking the autocleavage processing of pro-CatB. BAG2 control assembly of pro-CatB/annexin II complex to transport pro-CatB-containing vesicles toward the cell periphery, ultimately resulting in the secretion of pro-CatB, which triggers metastasis. Thus, BAG2 is a crucial regulator of the oncogenic role of pro-CatB in highly aggressive BC (Yang et al., 2017).

CatB secretion and activity regulation in TNBC involves the cell surface V-type ATPase. Inhibiting V-type ATPase with a specific inhibitor called concanamycin A decreases the activity of CatB in both the conditioned media and cell lysate (Uhlman et al., 2017). Another V-type ATPase-specific inhibitor, archazolid, has been shown to hinder the maturation of CatB, which results in reduced levels of intracellular mature CatB protein and ultimately decreased CatB activity. This inhibition occurs through the prevention of mannose-6-phosphate receptor-dependent trafficking. These studies indicate that V-type ATPase post-transcriptionally regulates CatB activity (Kubisch et al., 2014).

3. CatB in the delivery of breast cancer therapeutics

Recently, a considerable focus has been on researching and developing drug delivery systems that target malignant lung metastasis in BC patients. The studies have used a strategy like a linker for cleavage of prodrug by CatB for targeted delivery of antibody-drug conjugates (ADCs) (Table 8.1). For example, linkers present in trastuzumab duocarmazine (SYD985) as ADCs affecting HER2 can be cleaved by CatB. Lipophilic drugs that exhibit a bystander effect and eliminate adjacent antigen-negative cells are also employed. In patient-derived xenografts of BC, combinatorial treatment with inhibitors of CDK4/6 and SYD985 exhibited enhanced anticancer effects, suggesting the role of CatB in targeted drug delivery (Duro-Sánchez et al., 2022).

Drug−drug nanoparticles (DD-NPs) were synthesized using a CatB-cleavable peptide (FRRG) as a linker to conjugate doxorubicin (DOX) with a proapoptotic peptide drug (SMAC; AVPIAQ). The resultant DD-NPs were selectively cleaved in CatB-overexpressing cancer cells, releasing both SMAC and DOX to induce a synergistic anticancer activity. In a mouse model of MCF-7 tumors, DD-NPs administered via intravenous injection showed substantial accumulation in tumor tissues, thereby releasing SMAC and DOX and producing a synergistic anticancer effect. Furthermore, the metastatic lung cancer model exhibited a significant reduction in tumor growth as well as improved overall survival due to the highly effective DD-NPs. Therefore, CatB-dependent delivery of DD-NPs holds great potential as promising for treating cancers with drug resistance (Shim et al., 2020). Wang et al. developed a theranostic system for treating TNBC using a versatile nanoprobe (Pep-SQ@USPIO) and a sequential strategy. The system also targeted fibronectin using the Cys-Arg-Glu-Lys-Ala (CREKA) peptide for MR imaging and CatB-activatable fluorescence imaging, followed by enhanced photodynamic therapy (PDT) of TNBC.

The system used fibronectin overexpression in the extracellular matrix of TNBC as a biomarker. The self-developed squaraine photosensitizer (SQ) and ultrasmall superparamagnetic iron oxide (USPIO) were used for fluorescence and PDT, respectively. TNBC-derived CatB cleaved the Gly-Phe-Leu-Gly (GFLG) peptide, allowing the liberated SQ photosensitizer to enable fluorescence imaging and enhance PDT. This study shows that endogenous enzyme-activated nanoprobes and tumor-ECM-targeting can pave the way for TNBC theranostics (Wang et al., 2020). MORAb-202 is an antibody−drug conjugate that targets folate receptor alpha (FRA), which is commonly overexpressed in tumors. It consists of farletuzumab and eribulin linked by CatB-cleavable linker. MORAb-202 was effective in killing FRA-

TABLE 8.1 Examples of targeted drug delivery systems for treating breast cancer lung metastasis.

Strategy	Mechanism of action	References
Targeted delivery of ADCs using a CatB-cleavable linker, combined with CDK4/6 inhibitor	Enhanced anticancer effects observed in patient-derived xenografts of BC	Duro-Sánchez et al. (2022)
Synthesis of drug-drug nanoparticles (DD-NPs) using a CatB-cleavable peptide (FRRG) as a linker to conjugate doxorubicin (DOX) with a proapoptotic peptide drug (SMAC; AVPIAQ)	• Selective cleavage by CatB-overexpressing cancer cells and release of both SMAC and DOX-induced synergistic anticancer activity in a mouse model of MCF-7 tumors. • Showed substantial accumulation in tumor tissues and reduced tumor growth in metastatic lung cancer model	Shim et al. (2020)
Development of a theranostic system for the treatment of TNBC using a versatile nanoprobe (Pep-SQ@USPIO) and a sequential strategy	• Targeted fibronectin using the Cys-Arg-Glu-Lys-Ala (CREKA) peptide for MR imaging and CatB-activatable fluorescence imaging, followed by enhanced photodynamic therapy (PDT) of TNBC • Endogenous enzyme-activated nanoprobes and tumor-ECM-targeting paved the way for TNBC theranostics	Wang et al. (2020)
Targeting folate receptor alpha (FRA) using antibody-drug conjugate (MORAb-202) consisting of farletuzumab and eribulin linked by CatB-cleavable linker	• Effective in killing FRA-positive cells after cleaved by CatB; durable effectiveness positively correlated to tumor FRA expression in patient-derived xenograft model of TNBC	Furuuchi et al. (2021)
Synthesis of dual-sensitive block copolymer targeting CatB and conjugated with DOX, loaded with nifuroxazide (NFX) to self-assemble co-prodrug-loaded micelles (CLMs)	• Enzymatically biodegradable and significantly reduced viability, migration, and invasion of 4T1 murine BC cells in vitro • Showed enhanced antitumor efficacy and significant antimetastatic effects in orthotropic and lung metastasis 4T1 BC mice models, with decreased expression of matrix metalloproteinases and a decrease in the infiltration of MDSCs contributing to the inhibition of pulmonary metastasis	Luo et al. (2020)
Gold nanorods functionalized with CatB cleavable peptide and DOX.	• Reduction of drug resistance index • Significant reduction in cell viability	Zhi et al. (2019)
Stimuli-responsive dendritic polyHPMA-GEM	• Reduction of CatB • Significant antitumor activity and inhibition of tumor growth	Dai et al. (2018)

positive cells after being cleaved by CatB. In vivo studies showed that MORAb-202 had durable effectiveness positively correlated to tumor FRA expression in the patient-derived xenograft model of TNBC. MORAb-202 is a promising investigational ADC for treating TNBC (Furuuchi et al., 2021).

A dual-sensitive block copolymer targeting CatB was synthesized and then conjugated with DOX. The copolymer-DOX was loaded with nifuroxazide (NFX) to enhance its efficacy in self-assembly co-prodrug-loaded micelles (CLMs). In vitro studies showed that CLM was enzymatically biodegradable, with drug release being stimulated by pH/enzyme dual stimuli. It significantly reduced the viability of 4T1 murine BC cells and inhibited their migration and invasion. Upon i.v. injection of CLM in mice, the nanoscale size, and stimuli responsiveness of CLM facilitated drug delivery to the tumor site. In orthotropic and lung metastasis 4T1 BC mice models, CLM demonstrated enhanced antitumor efficacy and significant antimetastatic effects. Examinations of the tissues showed a considerable amount of programmed cell death, decreased expression of matrix metalloproteinases, and a decrease in the infiltration of MDSCs, all of which contributed to the inhibition of pulmonary metastasis. These findings suggest that CatB-cleavable CLM may have the potential for the delivery of nanomedicine to treat BC metastasis (Luo et al., 2020).

Treating multidrug-resistant BC cells using AU nanorods functionalized with a CatB-cleavable peptide and doxorubicin (AuNR-LAX-DOX) significantly reduced the drug resistance index of DOX-resistant MCF-7/ADR cells from 955.0 to 1.7. Additionally, the viability of MCF-7/ADR cells was significantly reduced after the treatment. Importantly, it showed 50% viability against the human noncancerous bronchial epithelial cell line 16HBE, whereas the viability of MCF-7 cells was close to zero. This study suggests that AuNR-LAX has the potential to curtail the harmful effects of DOX on normal cells and tissues and shows promise in conquering MDR while reducing harmful effects on normal cells (Zhi et al., 2019).

Using the one-pot method, Dia et al. synthesized a nanoscale system (dendritic polyHPMA-GEM) comprising a durable prodrug by linking gemcitabine (GEM) to dendritic polyHPMA copolymer. The prodrug showcases drug release characteristics that respond to enzymes, with more than 95% of GEM being discharged from the carrier in the presence of CatB. The cellular uptake of the dendritic prodrug and its ability to induce cell apoptosis coincide with its cytotoxicity. Furthermore, the prodrug accumulates highly within tumors, leading to significant antitumor activity in the 4T1 murine BC model. The drug released from prodrug significantly suppressed the tumor volumes indicating inhibition of tumor growth. Further, IHC analysis of the tumor xenograft suggests that cell death occurs via antiangiogenic effects, indicating that the stimuli-responsive dendritic polyHPMA-GEM has significant anticancer activity (Dai et al., 2018).

4. Potential of cathepsin B inhibitors for breast cancer treatment

CatB is essentially involved in normal physiological processes, but its abnormal expression has been implicated in different pathological conditions, including cancer. As a result, inhibitors targeting CatB have emerged as potential therapeutic agents for cancer treatment. Several inhibitors, small molecules, peptides, and antibodies that target CatB activity and its interaction with other proteins and trafficking have been developed and studied in preclinical settings. The development of effective and selective CatB inhibitors remains an active area of research, with promising potential for improving the treatment of BC. Oblongifolin C (OC) belongs to the polycyclic polyprenylated acylphloroglucinol class of compounds

and is derived from *Garcinia yunnanensis* Hu. Studies have demonstrated that OC triggers cell death apoptosis, restrains autophagic flux, and prevents cancer metastasis. In addition, both surface plasmon resonance (SPR) and isothermal titration calorimetry (ITC) experiments suggest that OC can interact with HSPA8 and CatB, ultimately inhibiting HSPA8 nuclear translocation and CatB activities. These findings suggest that OC could be developed as a promising anticancer drug (Han et al., 2020).

Amentoflavone (AMF1), a natural biflavone compound obtained from *Taxodium mucronatum*, and hinokiflavone, isolated from *Cycas guizhouensis*, are potent inhibitors of CatB, with IC_{50} of 0.62 and 0.58 μM, respectively. According to inhibition and flexible docking studies, these biflavones are reversible inhibitors of CatB, and their binding patterns and interaction modes with CatB make them more specific to CatB endopeptidase (Zeng et al., 2006). Seahorse (Hippocampus kuda Bleeker) contained three previously unknown phthalate acid derivatives, namely 2,12-diethyl-11-methylhexadecyl 2-ethyl-11-methylhexadecyl phthalate, 2-ethyldecyl 2-ethylundecyl phthalate, and bis(2-ethyldodecyl) phthalate. These compounds were observed to inhibit catB activity in a dose-dependent manner, with IC_{50} values of 0.13, 0.21, and 0.18 mM, respectively. Besides, limited natural and synthetic inhibitors of CatB are explored for BC treatment (Table 8.2).

4.1 Natural CatB inhibitors against breast cancer

The potential anticancer natural flavonoid decursinol angelate (DA), thymol (TH), and propionic acid derivative ibuprofen (IB) were evaluated for their ability to inactivate CatB using enzymatic assay, computational modeling, and in vitro methods. Among these compounds, DA was the most promising candidate for inhibiting CatB, with the lowest IC_{50} value observed after 1 hour of incubation with Z-Phe-Arg-4MβNA (BANA) as a substrate. Docking analysis further revealed that DA interacted favorably with the catalytic site residues (GLN23, CYS26, HIS110, HIS111) of CatB (PDB Id: 1HUC), which accounted for its ability to inhibit the proteolytic activity of the enzyme. In vitro quantification with human cancer cells also demonstrated that DA rapidly inactivated CatB, outperforming the commercial synthetic inhibitor CA074, without causing any cellular toxicity to normal cells (Sharma and Kang, 2022).

According to Gianotti et al., CaneCPI-4, a cystatin derived from sugarcane (*Saccharum officinarum*), exhibited potent inhibitory activity against CatB (K(i) = 0.83 nM). CaneCPI-4 expression in MDA-MB-231 cells only slightly reduced their invasive ability. However, when low levels of recombinant His-tagged CaneCPI-4 were added, migration through a Matrigel was significantly reduced without causing toxicity. Immunoblot analyses revealed that endocytosis did not internalize the recombinant protein, suggesting that the cystatin triggered its antimigratory effect by inhibiting secretory CatB (Gianotti et al., 2008). According to Cecarini et al., treatment with the cyanogenic glycoside amygdalin from apricot kernels reduced 20S and 26S proteasome activities after 24 h. This treatment also impaired CatB activity, decreasing cancer cell migration, autophagy, and activation of apoptotic events in MCF7 cells (Cecarini et al., 2022). According to Calaf et al., the administration of curcumin resulted in a decrease in CatB levels in tumor 2 cells. Conversely, curcumin enhanced the expression of E-cadherin, c-myc, and CD44 in the MCF-10F immortalized human breast

TABLE 8.2 Therapeutic strategies targeting cathepsin B (CatB) for treatment of breast cancer.

Therapeutic strategy	Targeted effect on CatB	Efficacy in preclinical models	References
Decursinol angelate (DA)	• Inhibition of CatB activity.	In vitro cell line model	Sharma and Kang (2022)
CaneCPI-4	• Inhibition of secretory CatB. • Reduced migration of BC cells.	In vitro cell line model	Gianotti et al. (2008)
Amygdalin	• Inhibition of CatB activity. • Reduced cell migration MCF-7 cells.	In vitro cell line model	Cecarini et al. (2022)
Curcumin	• Decreased CatB levels.	Tumor 2 cell model	Calaf and Roy (2017)
Rhenium(I)-diselenoether complex	• Reduction of CatB production. Significant decrease in cell viability.	In vitro cell line model	Collery et al. (2021)
Ruthenium compounds with nitroxoline derivatives.	• Effective inhibition of CatB. • Decline in ECM degradation and invasion of breast epithelial cells.	Transformed breast epithelial cells	Mitrović et al. (2019)
Synthetic analog of peptidyl epoxide, CA-074	• Specific inhibition of CatB. • Significant decrease in metastatic spread.	In animals with established tumors	Withana et al. (2012)
Triazole-based selective reversible inhibitor of cathepsin X named Z9 (1-(2,3-dihydrobenzo [b] [1,4]dioxin-6-yl)-2-((4-isopropyl-4H-1,2,4-triazol-3-yl)thio)ethan-1-one)	• Synergistic effect with CatB inhibition. • Reduction in tumor progression.	In vitro in cell-based functional assays and in vivo tumor mouse models	Mitrović et al. (2022)
Anti-CatB antibody inhibitor	• High specificity in inhibiting CatB. • Strong efficacy and selectivity for human CatB	In vivo mice model	Dai et al. (2020)

epithelial cell line. The findings suggest that curcumin can potentially affect cancer progression and may be a viable therapeutic option for advanced BC patients without the side effects commonly associated with traditional therapeutic drugs (Calaf and Roy, 2017).

4.2 Synthetic inhibitors of cathepsin B for breast cancer

Rhenium(I)-diselenoether (Re-diSe) is a promising drug candidate for metastasis treatment, which exhibits specific inhibitory effects on TNBC cells. At a low dose (10 μM), Re-diSe significantly reduces the CatB levels and the viability of cancer cells (Collery et al., 2021). Another study has demonstrated that ruthenium compounds containing nitroxoline or its derivatives, which are potent and selective reversible inhibitors of CatB, can effectively inhibit the activity of CatB. This results in a decline of ECM degradation as well as invasion of breast epithelial cells transduced with oncogenes, suggesting that these compounds may hold promise for the treatment of BCs mediated by CatB (Mitrović et al., 2022) Additionally, administration of the highly selective inhibitor CA-074, which specifically targets CatB, via the intraperitoneal route resulted in a significant decrease in the metastatic spread in animals with established tumors (Withana et al., 2012).

The tumor progression was reduced by a reversible inhibitor of cathepsin X named Z9, which is based on triazole. The inhibitor showed significant results both in vitro and in vivo, as it was tested in two independent mouse models. One of the ways by which cathepsin X contributes to cancer progression is by compensating for the loss of CatB activity. Inhibition of both CatB and X showed a synergistic effect, which can help overcome resistance to antipeptidase therapy. Thus, simultaneous inhibition of both cathepsins could be a promising approach to impair CatB-directed tumor progression (Mitrović et al., 2022).

A genetically engineered humanized antibody inhibitor targeting human CatB was developed by fusing the propeptide of pro-CatB, a natural inhibitor of CatB, into the complementarity-determining region 3 of the heavy chain (CDR3H) of Herceptin. This antibody—propeptide conjugate exhibited high specificity in inhibiting CatB proteolytic activity at nanomolar concentrations. Moreover, investigations into its pharmacokinetics in mice revealed a plasma half-life of about 42 h, indicating its strong efficacy as well as selectivity for human CatB. Therefore, this anti-CatB antibody—peptide inhibitor can be potentially helpful in treating CatB-mediated BCs (Dai et al., 2020). These findings suggest that CatB plays a critical role in developing bone metastasis and inhibiting it could be a promising therapeutic strategy for managing metastatic BC.

5. Conclusion

In conclusion, metastasis is a severe complication in BC patients, and comprehending the mechanisms that facilitate its progression is essential for developing efficient therapeutic approaches. Evidence has demonstrated that CatB contributes to the transformation of breast epithelial cells, resulting in premalignant progression by promoting the degradation of ECM components. Research has established that CatB is associated with an unfavorable prognosis, lymph node infiltration, and distant metastasis in BC patients. Recently, several natural and synthetic compounds were explored as CatB inhibitors in preclinical and clinical models. CatB is also explored as linker peptide cleavage for targeted drug delivery. Hence, CatB holds promise as a potential candidate for both prognostication and therapy in managing metastatic BC.

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CHAPTER

9

Kallikrein-related peptidase 3 (KLK3) as valuable biomarkers for prostate cancer

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1. Prostate cancer

Prostate cancer (PC) is the most common cancer diagnosed and the fifth leading cause of cancer death in men (Leslie et al., 2024). Factors such as increased age, black race, and family history of the disease are few of the risk factors responsible for PC (Platz and Giovannucci, 2009). By 2040, it is anticipated that the incidence of PC would approach 2.3 million, and nearly 740,000 people will succumb to this cancer (Culp et al., 2020). Genetic risk shows the evidence why it is more common in Africans compared with other races. Exposure to chemicals, fertilizers, pesticides, and environmental contaminants are associated risk factors. Increased saturated fat and red meat intake result in high risk of PC in individuals as compared with those with low intake of these food items (Hayes et al., 1999). Consumption of eggs, dairy products, and calcium were reported to be risk factors (Wu et al., 2016). A Western diet may increase the risk for PC compared with a Mediterranean diet, which may be more beneficial (Jalilpiran et al., 2018).

Prostate is a fibromuscular male reproductive organ, which is sensitive to many hormonal changes. Testicular androgen plays an important role in the growth, development, and the function of the prostate gland. Benign prostatic hyperplasia is associated with the production of testicular hormone.

Majority of the PCs are adenocarcinomas, which develop in the gland cells, and the ductal type is very rare. Both adenocarcinomas and ductal types may be differentiated histologically. Some of the common clinical features include painful micturition, hematuria, and urinary obstruction. The prognosis of ductal type of PC is bad compared with the acinar adenocarcinomas.

Testosterone was reported to modulate apoptotic processes in hormone-dependent prostate cells by affecting the expression level of apoptotic genes and, thus, promoting cancer

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development. Prostate tumors were found to express androgen receptors (ARs), and androgen deprivation therapy was found to be beneficial in reducing tumor progression (Vickman et al., 2020). Also, there is difference in opinion regarding androgen receptor therapy being linked to PC.

Various pathways are involved in the pathogenesis of PC. The phosphatidylinositol-3-kinase (PI3K), protein kinase B (PKB/AKT), and mammalian target of rapamycin (mTOR) pathway were found to be involved in PC (Shorning et al., 2020). The PI3K-AKT-mTOR signaling network, which is involved in tumorigenesis, progression, and recurrence of PC, needs to be further elucidated. Hence, PI3K-AKT-mTOR pathway inhibitors can be effective as therapeutic agents. In PC, the aberrant activation of Wnt pathway is a common event, which is responsible for tumor formation, progression, and drug resistance (Koushyar et al., 2022). WNT/β-catenin signaling has an important role in castration-resistant PC patients. Therapeutic targets against Wnt pathway can be beneficial for treating PC patients.

2. Diagnosis of prostate cancer

Many slowing growing tumors are asymptomatic for long time. Prostate-specific antigen (PSA) refers to a protein, which is produced by cells in the prostate gland, and it is found in the semen and bloodstream. In any pathological condition related to the development or growth of prostate, more PSA is released and can be detected in the blood. The malignant condition is that the PSA penetrates the cell wall into the extracellular fluid and ultimately into the blood. In conditions such as hyperplasia, inflammation, and infection, PSA may also increase.

PSA sensitivity ranges between 9% and 33%, it depends on age, and the PSA cutoff values used (Leal et al., 2018). This means that up to 91% of individuals with elevated serum PSA levels do not even have PC (Leal et al., 2018). Interestingly, it has been seen that in clinical practice, the actual risk of PC in men with PSA elevated levels is approximately 30% (Leal et al., 2018). According to the National Cancer Institute, the normal or abnormal level of PSA in the blood remains unknown because few individuals with PSA levels below 4.0 ng/mL had prostate cancer, while others with higher PSA levels ranging between 4 and 10 ng/mL did not have prostate cancer (Thompson et al., 2004).

Unfortunately, 20% of PC patients who are labeled as a low risk, using the criteria of PSA level, stage of the tumor, and Gleason score, die during conservative therapy, and this underlines the importance of advanced biomarkers to detect the aggressive stage (Lin et al., 2021). The diagnosis for PC is done based on physical digital rectal examination (DRE), measurement of PSA in the blood, radiological imaging (ultrasound and magnetic resonance imaging), and biopsy obtained from the prostatic tissue. Kallikrein regulates the formation PSA, which is a diagnostic tool in PC cases (Fig. 9.1).

From Pro-PSA to PSA, the conversion is regulated by kallikreins. PSA is then expressed in prostate cancer and is used as a valuable marker.

Gleason grading is done to know the severity of the tumor. A Gleason score of 6,7, and higher than 8 denotes low-grade, intermediate-grade, and high-grade cancer, respectively.

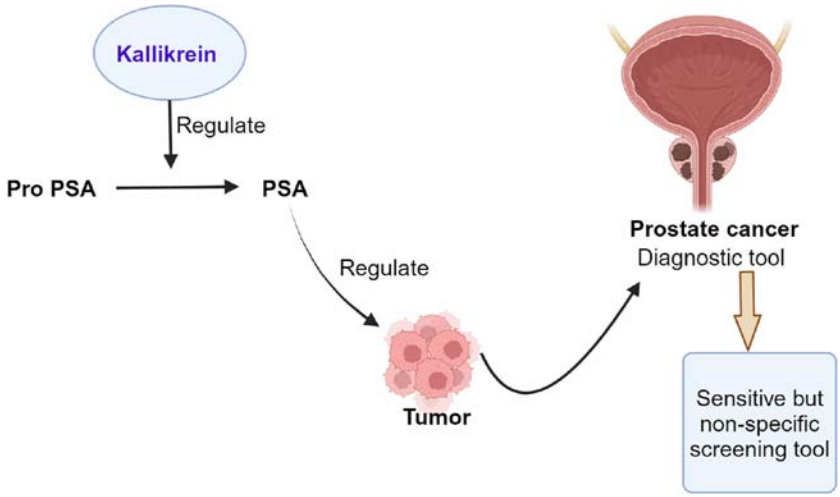

FIGURE 9.1 Schematic diagram showing how kallikrein regulate the PSA, which is a diagnostic tool in PC cases. This figures describes the formation of prostate-specific antigen and its usefulness as a diagnostic marker in prostate tumors. Created with BioRender.com.

In the year 2014, International Society of Urological Pathology released a revised grading system for the PC, which was known as the "Grade Groups." The biopsy tissue viewed under the microscope helps in knowing the "Grade Groups." The spread of the disease can be known by bone scan and radiological imaging (magnetic resonance imaging and positron emission tomography scan).

3. Kallikreins as important serum biomarkers

In the early 1930s, Kraut, Frey, and Werle (Kraut et al., 1930) showed that a substance was abundant in pancreatic extracts; hence, it was named "kallikrein," which was synonymous to the Greek word for pancreas, kallikreas (Kraut et al., 1930; Werle et al., 1937). Kallikreins (KLKs) are serine proteases, which are involved in different physiological processes. There are two isoforms of the kallikrein protease, i.e., plasma kallikrein (produced in the pancreas and circulate in the blood) and tissue kallikrein (which are expressed in different tissues of the body) (Kalinska et al., 2016).

The hormones and steroids regulate the expression of KLKs. The KLKs are important markers of the hormone (Shang et al., 2014) receptor activity. KLKs have been implicated in various cascade enzyme pathways. The kallikrein−kinin system is a metabolic cascade, which when activated releases kinins. This cascade depends on signaling via two cross-talking receptors, i.e., bradykinin receptor 1 (B1R) and bradykinin receptor 2 (B2R) (da Costa et al., 2018). Signaling by kinins B1R and B2R leads to production of nitric oxide, increase in vascular permeability, and angiogenesis. The tumor cells invade the stromal tissue where there is more vascular permeability. Increase in vascular permeability helps in cancer metastasis and induces inflammatory responses, which contribute to tumor growth.

The tissue KLK consisting of 15 proteases was mapped to genes located on chromosome 19q13.4 (Yousef et al., 2001), while plasma KLK, encoded by the *KLKB1* gene, had the location on chromosome 4q34−35 (Kalinska et al., 2016). Both plasma and tissue KLKs differ in terms of their molecular weight.

In many tissues regulated by hormones such as testis, breast, and prostate, kallikreins are expressed. Kallikreins are involved in tissue modeling, growth of cell, and angiogenesis. In PCs, many KLKs expressions are dysregulated.

Kallikrein gene family has three genes, i.e., *KLK1* (encoding human kallikrein 1 [hK1]), *KLK2* (encoding hK2), and *KLK3* (encoding hK3 or PSA) of which *KLK2* and *KLK3 have an important association with PC* (Diamandis et al., 2000).

PSA, also termed as kallikrein-related peptidase (KLK)-3 is a PC marker, which has been commonly used. Earlier, prostatic acid phosphatase (PAP) was used to ascertain the success or failure of hormone therapy, but later, use of PAP for early detection of PC was not too successful (Lowe and Trauzzi, 1993).

Because of the similarity of structure in between PSA and other KLKs, the role of other members of KLK family as a useful sensitive and specific biomarker for cancer of the prostate were also investigated during the past 25 years (Hong, 2014). Even being similar in structure, KLKs and PSA differ in their enzymatic action. There are many KLKs that are expressed in the prostate and are regulated by androgens such as KLK3 (PSA) (Lawrence et al., 2010).

Kallikrein-related peptidase 2 (KLK2), encoded by the KLK2 gene, is mainly expressed in the prostate tissue. It is located in the prostatic epithelium. KLK2 may be found together and expressed together with KLK3. In approximately 1% of the concentration of PSA, KLK2 may be present in seminal plasma (Lövgren et al., 1999), and based on in vitro studies, KLK2 functions as a protease to activate itself (Kumar et al., 1997). The urokinase plasminogen activator (uPA) converts the proenzyme plasminogen to the active serine protease plasmin (responsible for degradation of extracellular matrix), and KLK2 was found to activate the uPA (Solovyeva et al., 1996).

Androgen controls the expression of KLK2 gene, and it has been noticed that the levels of the (mRNA) *KLK2* were significantly increased by mibolerone and dihydrotestosterone (Young et al., 1992). In castration-resistant PC, it was found that increased KLK2 expression correlated with higher cell proliferation rate and lower cell apoptosis index (Young et al., 1992).

The level of expression of KLK5 and KLK7 are useful in determining the aggressiveness of the PC. There is overexpression of KLK5 in normal against PC tissue, and moreover with an increase in pathological stage of the tumor, an inverse relationship in the level of KLK5 was reported (Kurlender et al., 2004). Interestingly, KLK5 may be differentially expressed in various endocrine tumors. There are reports of KLK5 being overexpressed in ovarian cancers (Diamandis and Yousef, 2001) while its level decreased in PC when compared with normal tissue (Yousef et al., 2002). The role of hormones plays an important role in the regulation of the kallikreins.

KLK6 mRNA levels are associated with cancer of colon. Studies reveal that knockdown of KLK6 in colon cancer cells results in suppression of the invasive and metastatic nature of the cancer (Sells et al., 2017). The phosphoinositidine 3-kinase (*PIK3CA*) is commonly mutated gene in colorectal cancer, and it has been detected in 43% of the KLK6-high tumors (Pandey et al., 2021).

In malignant conditions of the prostate, *KLK2, KLK4, KLK11*, and *KLK14-15* are expressed (Fuhrman-Luck et al., 2014). KLK3 is an important serum biomarker in PC patients. Researchers have reported that higher kallikrein-related peptidase 4 (KLK4) mRNA levels in prostate biopsy correlated to higher Gleason score and stage (Avgeris et al., 2010). Three *KLK3* SNPs, i.e., rs17632542, rs62113212, and rs2569735, and 3145 SNP interaction pairs were found to be associated with aggressiveness of PC (Lin et al., 2021).

KLK4 expression was reported to be associated with increased risk of PC and stage of the tumor (Avgeris et al., 2011). Kallikrein 4 (*KLK4*/hK4) has gained considerable interest as *KLK4* mRNA was shown to be elevated in individuals with PC (Veveris-Lowe et al., 2005). hK4 is confined to the cytoplasm of glandular epithelial cells and highly expressed in a cancer tissue compared with a benign tissue (Veveris-Lowe et al., 2005). KLK4 and KLK2 were reported to be highly associated with PC compared with PSA (Veveris-Lowe et al., 2005). Interestingly, KLK4 may be more important than PSA in the motility of cancer cells and cancer progression (Veveris-Lowe et al., 2005).

hK4 has been implicated in the bone metastasis spread of PC. hK4 may be seen both in the cells of the tumor and in the osteoblasts. The expression of hK4 in the cells of the PC was induced by osteoblasts in both a direct and indirect manner (Gao et al., 2007). This gives the evidence of potential interaction between the cells of PC and the osteoblasts.

The expression of *KLK5* was shown to be inversely correlated with malignant prostatic tumor and Gleason score (Yousef et al., 2002). DNA methylation of *KLK10* showed a positive correlation with pathological stage of the disease (Olkhov-Mitsel et al., 2012).

The expression of *KLK14-15* correlates to the pathological stage (Yousef et al., 2003), while *KLK11* increased in PC and inversely related to the stage and grade of the tumor (Nakamura et al., 2003). The newly discovered *KLK15* gene is located adjacent to *KLK3* on chromosome 19q13.4 and has 41% similarity to *KLK3* (Yousef et al., 2002). The KLK1 and KLK3 genes surround the KLK15, which is expressed and synthesized as an inactive zymogen (pro-KLK15) (Yousef et al., 2001). The expression of KLK15 is upregulated by steroid hormones at the mRNA level in PC (Yousef et al., 2001). KLK15 is associated with the more aggressive stage of the cancer. A research study in Germany conducted on prostate tissue samples obtained from 29 patients showed that overexpression of KLK15 is associated with the severity of the PC and implies bad prognosis (Yousef et al., 2001).

4. Clinical trials and case control studies

There is an ongoing clinical trial on subjects aged above 18 years, aimed to determine the recommended phase 2 dose(s) (RP2D[s]) of JNJ-69086420 in Part 1 (Dose Escalation) and to determine safety and initial signs of clinical activity at the RP2D(s) in Part 2 (Dose Expansion) 9 (Clinical trial/NCT04644770, 2020). In this study, JNJ-69086420, an actinium-225-labeled antibody targeting the hK2 is being studied in PC. This clinical trial has all those with metastatic castration-resistant prostate cancer (mCRPC) with histological confirmation of adenocarcinoma (adenocarcinoma with small cell or neuroendocrine features), and having prior exposure to at least one androgen receptor (AR)−targeted therapy was being included (Clinical trail/NCT04644770, 2024).

A study in Sudan aimed to study the validity of serum kallikrein-2 (KLK-2) and free prostate-specific antigen (fPSA) for early detection of PC in 70 men who were diagnosed with PC (Yousif et al., 2023). In the same study, the control group consisted of 60 benign hyperplasia of prostate (BPH) and 45 healthy men, and the blood samples were measured for KLK-2, fPSA, and PSA (Yousif et al., 2023). The results showed elevated PSA, fPSA, and KLK-2 among the study groups and concluded that KLK-2/PSA and KLK-2/fPSA ratios enhance sensitivity and specificity of detecting PC cases compared to using KLK-2, fPSA, and PSA alone (Yousif et al., 2023). Thus, instead of measuring KLK-2 or fPSA alone, it is advisable to measure KLK-2/fPSA ratio.

A study in Canada measured the changes in the level of serum kallikrein (KLKs) levels during intensity-modulated radiotherapy (IMRT) in PC patients and looked at the possible correlations between kallikrein level in the serum and normal tissues toxicity during radiation (Nasser et al., 2017). The results of the study showed that KLKs 2, 3, 6, and 11 decreased significantly after the completion of definitive radiation therapy. The KLK11 increased during radiation, due to acute cell injury causing spillage of KLK11 into the serum, and then, decreased 1 year following definitive radiation therapy compared with baseline levels. Almost halfway through the radiation course, the KLK2 and intact PSA serum levels also dropped (Nasser et al., 2017). The results show that KLKs level changes during and after radiation therapy.

A study conducted in Germany, which looked into association of total and free forms of serum hK2 and PSA with PCs of unfavorable prognosis in a cohort of 867 men, showed the ratio of fhK2 to thK2 slightly decreased from a median of 88% in organ-confined tumors to 78% in cancers with seminal vesicle invasion (Steuber et al., 2007).

Another recent case control study was conducted in Helsinki in which 79 patients with localized grade group 2−4 aggressive cases of PC (with metastasis), and 86 patients with similar baseline characteristics (no metastasis) were taken as cases and controls, respectively (Lehto et al., 2023). The results showed that the expression of many KLKs including KLK12 was increased in cancer, but on the contrary, the expression of KLK2-4 and -15 was decreased in aggressive PCs compared with controls (Lehto et al., 2023). Elevated KLK14 mRNA and protein levels were reported to be associated with aggressive conditions of the tumors (Emami et al., 2008).

Total PSA (tPSA) and fPSA are important markers in PC, but they have low specificity. A prospective validation study was conducted in the United States, and it examined the 4Kscore Test in 1012 men to find out the accuracy of the test to predict the probability of high-grade cancer on biopsy (4Kscore Test, 2022). The FDA approved the 4Kscore Test, and its accuracy has been tested by various researchers.

A multiinstitutional prospective trial was conducted in the United States using in predicting Gleason ≥7 PCa in the United States (Parekh et al., 2015). Based on the results, it was concluded that 4Kscore is a useful tool in differentiating between individuals who are likely to have clinically relevant cancer and those who are likely to have indolent tumors or without cancer. The researchers held the view that the 4K test has enough potential to reduce the number of biopsies as seen from the results, where it was seen that 36%−82% biopsies could be avoided or delayed (Parekh et al., 2015).

A systematic review, which used PubMed and Embase databases (from 1980 to 2011), found that up to 60% of PC diagnosed in contemporary studies can simply be observed

without the need for immediate intervention (Dall'Era et al., 2012). Most malignancies found because of elevated PSA levels are in fact low-risk PCs, which are often managed by monitoring with active surveillance (David and Leslie, 2024). Active surveillance may be important, rather than subject the patient to overdiagnosis methods. The role of biopsy has been given considerable interest.

The 4Kscore test may reduce unnecessary decision for a biopsy to be performed in PC cancers. A multiinstitutional prospective trial was conducted in the African region (Punnen et al., 2018). The results showed that the 4Kscore test was accurate for predicting the aggressive prostate cancer for making any decision in African American and non-African American men. Again, it was concluded that 22% of the subjects studied could avoid or delay the biopsies (Punnen et al., 2018).

There is a school of thought that unless fPA to tPSA ratio is low, a PC patient may not need biopsy. The Malmö Diet and Cancer study (1991–96) was conducted on a population-based cohort of 11,063 Swedish men who were aged between 45 and 73 years and blood sample was used at baseline (Vickers et al., 2011). It was concluded that using tetrad of four kallikrein markers (total PSA, free PSA, intact PSA, and hK2), it would be more highly accurate predictor and thus determine whether biopsy needs to be done or not. The FDA has also approved the 4K score.

5. Conclusion

PSA is secreted by prostate gland in both normal and malignant state. PSA is measured in blood in ng/mL. Both PSA and hK2 share structural similarities. PSA is considered to be an important marker for PC, but it should be remembered that serum PSA is not only increased in PC but also in other conditions such as benign hyperplasia of prostate, inflammation, and infections. Expression of kallikreins may be regulated by steroid hormones. The inactive precursor form of PSA, known as proPSA, is rapidly converted to active PSA by hK2 and thus justifies the in vivo regulation of by PSA action by hK2. KLKs remodel the microenvironment of the tumor. KLKs act as biomarkers for the screening, diagnosis, and prognosis of cancer growth. The expression of kallikreins may correlate to the aggressiveness of PC. Association of kallikreins with PC paves the way for considering them as future therapeutic targets. KLKs, while being used as important biomarkers for diagnosing PC, may also help in deciding whether the PC patient must go for biopsy or not. Thus, it may help in curbing any unnecessary decision to get the biopsy done for the patient.

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CHAPTER

10

Ubiquitin specific protease 3,7 in bone cancer and squamous cell carcinoma

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1. Introduction

The bone constitutes a vital component of the human body, serving several roles, including facilitating movement, providing structural support, generating blood cells, and retaining essential minerals. Bone tissue remains dynamic in its metabolic activity, constantly undergoing remodeling and reconstruction processes throughout an individual's lifespan, effectively safeguarding crucial organs (Niedźwiedzki and Filipowska, 2015). Roughly five cases per million individuals represent the yearly occurrence of osteosarcoma (OS) within the general population. A combination of several medications given both before and following surgical surgery has been the recommended treatment approach for many years (Grünewald et al., 2020). Most proteins are modified in several ways after they are synthesized, including methylation, glycosylation, acetylation, sumoylation, phosphorylation, and ubiquitination (Heare et al., 2009). A little ubiquitin molecule is attached to certain lysine residues in proteins to cause ubiquitination, a major posttranslational modification. Various

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types of ubiquitination, such as poly-, mono-, and branching ubiquitination, are necessary for critical cellular processes such as controlling the cell cycle, reacting to DNA damage, reorganizing chromatin, and permitting proteasome-mediated protein degradation. Any alterations to this mechanism may have a role in the development of cancer. It is important to highlight that deubiquitinases (DUBs) are in charge of the reversible process of ubiquitination, which involves the removal of ubiquitin chains from certain proteins. This process regulates the stability and functionality of the targeted proteins (Meyers et al., 2008). As of the present, approximately 100 DUBs have been recognized as encoded by the human genome. An imbalance in protein synthesis or degradation may potentially contribute to the growth of primary tumors and the progression of metastasis. Thus, an increasing body of research suggests that ubiquitin-specific proteases (USPs) are vital in the modulation of tumor development, impacting key processes associated with cancer progression, such as apoptosis and cell division (Pal et al., 2014). Despite limited information on USPs and primary bone sarcomas, particularly OS, previous studies have indicated the inhibitory effect of USP1 on U2OS OS cells, where its overexpression has been linked to certain malignancies such as non−small-cell lung cancer. In vivo experiments have shown that the suppression of USP22 leads to a reduction in OS tumor growth and metastasis. Additionally, research has found that reducing USP39 expression suppresses the growth of osteosarcoma cells and induces apoptosis (Simpson and Brown, 2018). This review will explore the function of USP in bone cancer and SCC.

2. Ubiquitin

Despite Schlesinger's identification of ubiquitin in 1975, investigations into protein ubiquitination advanced during the 1980s. Numerous studies emphasize the crucial role of protein ubiquitination in controlling various cellular functions, including cellular positioning, function, interaction with other proteins, and degradation through the proteasome (Asmamaw et al., 2020). Research shows that the reversible process of ubiquitination is facilitated by enzymes called DUBs. Ubiquitin, a highly conserved protein found in all eukaryotes, acts as a marker for proteins requiring degradation. It has an average molecular weight of 8.5 kDa and consists of 76 amino acids, with two glycines crucially characterizing it. The C-terminus of ubiquitin plays a pivotal role in its function, especially in the covalent attachment to target proteins. Due to their critical role in controlling various cellular functions, including cellular homeostasis, any abnormal expression or activity of these enzymes, known as DUBs, may potentially contribute to the onset and progression of diseases such as cancer (Gianferante et al., 2017).

3. Ubiquitination

Ubiquitination, one of the posttranslational modifications (PTMs) linked to protein breakdown, is the process by which ubiquitin binds to specific sites on target proteins. Several enzymes play a role in ubiquitination including ubiquitin activators (E1), ubiquitin conjugators (E2), and ubiquitin ligators (E3). The illustration represents the process of ubiquitination (Fig. 10.1). The variety of these enzymes adds complexity to the ubiquitination process.

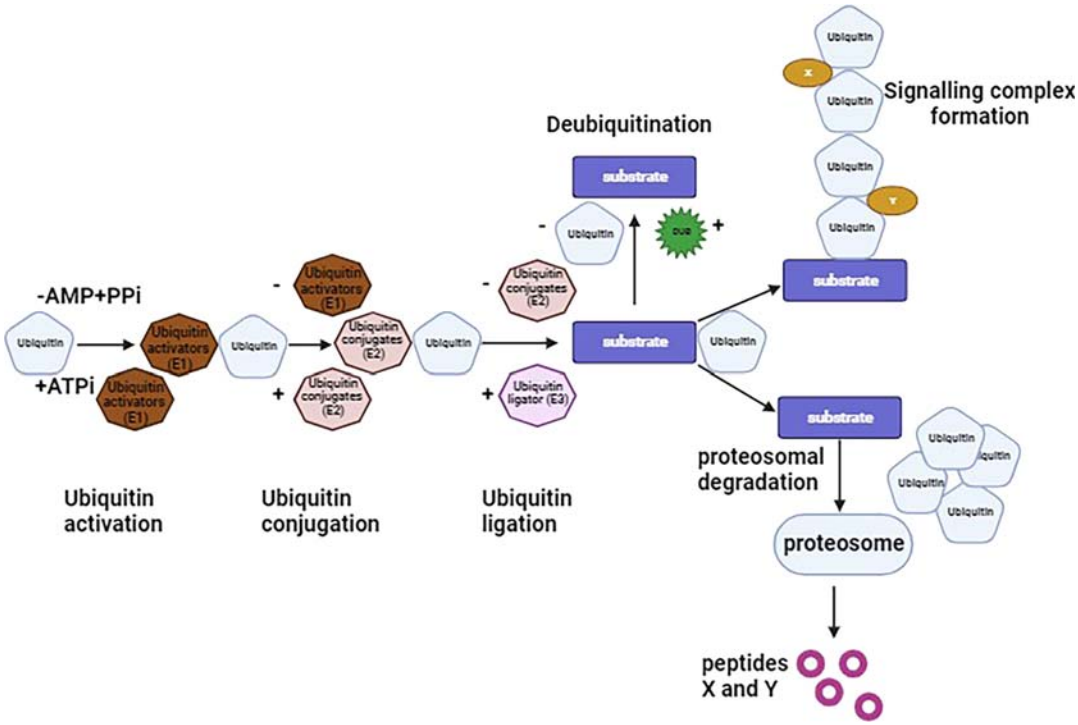

FIGURE 10.1 Process of ubiquitination and deubiquitination.

Each protein molecule can be modified by one or more ubiquitins. Specifically, monoubiquitination can be defined as the process of attaching a single ubiquitin molecule either to one lysine site on a protein or to multiple lysine sites on that protein. On the other hand, polyubiquitination entails the consecutive attachment of an individual ubiquitin molecule to a different lysine site on the ubiquitin molecule itself (Serrano et al., 2018). Ubiquitination is involved in the regulation of various essential biological mechanisms, including but not limited to protein breakdown, programmed cell death, genetic information expression, DNA damage restoration, and the immune response to inflammation, among others. The ubiquitination process affects over 80% of proteins and transpires in three stages: an early stage of ATP-dependent activation that is assisted by an enzyme called E1 activator, the ubiquitin molecule forms a connection with an E2 conjugating enzyme at a later step, and a last stage in which an E3 ligase covalently attaches the ubiquitin molecule to its target protein (An et al., 2015).

4. Ubiquitin-specific proteases

The largest group of DUBs is represented by the USP family, sometimes referred to as UBP in yeast. While yeast possesses 16 members of the USP/UBP family, it is estimated that

humans have over 50 such members. Among the identifying features of these enzymes are two short and conserved motifs known as the "histidine box" and the "cysteine box" that encompass the specific residues that are necessary for the proteolytic process of ubiquitin (Komander, 2009). These motifs are either found in single enzymes or combinations of enzymes. Among other activities, USPs are involved in carcinogenesis, inflammatory reactions, viral infections, and neurodegenerative illnesses. The USP family stands as the most extensive and varied group of DUBs, encompassing a total of 58 members (Nijman et al., 2005). USPs operate in diverse manners, displaying variations in sizes and structural domains. All USPs' catalytic core structures are identical, comprising three regions: cysteine residues, histidine residues, and aspartic/acyl-asparagine residues. Arranged akin to the palm of a hand, these domains form a catalytic triad that extends to interact with ubiquitin.

4.1 Ubiquitin-specific protease 7

Ubiquitin-specific protease 7 (USP7), alternatively referred to as herpes virus−associated USP (HAUSP), functions as a deubiquitylase crucial for overseeing vital cellular activities. It plays a significant part in regulating the functions of certain transcription factors, including p53, Foxp3, and β-catenin. USP7 has a strong affinity for interacting with MDM2, which consequently restricts the accumulation of p53 in cells not experiencing stress (Pal et al., 2014). Several studies have attempted to evaluate the impact of USP7 expression on cancer prognosis. However, the results have been inconclusive and conflicting. High levels of USP7 expression have been associated with poor outcomes in patients with neuroblastoma, colon tumors, breast cancer, hepatocellular carcinoma, multiple myeloma, cervical cancer, glioma, and cutaneous melanoma (Gan et al., 2017). According to Hernandez-Perez et al., both very high and very low levels of USP7 are correlated with unfavorable prognoses in breast cancer patients. Hence, according to clinical studies, USP7 may either facilitate or inhibit tumor development, contingent upon the specific type of cancer (Bousquet et al., 2016).

4.2 Ubiquitin-specific protease 3

Ubiquitin-specific protease 3 (USP3), found on the 15q22.3 region of the human chromosome, constitutes a part of the USP family, comprising 520 amino acids. It carries two essential histidines and a conserved cysteine residue that are essential for promoting the deubiquitylation process of particular target proteins (Kansara et al., 2014). USP3 controls the amounts of monoubiquitinated H2A and H2B, and when it is inhibited in U2OS cells, ionizing radiation-induced DNA damage is increased and S-phase delaying occurs. USP3 reverses the ubiquitin modification of H2A and Y-H2AX proteins in Hela cells following UV-triggered DNA damage. It does so by focusing on RNF168, leading to decreased levels of BRCA1 at locations where DNA breaks are present. In HepG2 cells, overexpressing the wild-type Usp3 deubiquitinates MDM2 and raises its protein levels. However, in HL-7702 normal liver cell lines, heightened USP3 expression does not stabilize MDM2. Coimmunoprecipitation (CO-IP) experiments suggest no interaction between USP3 and MDM2, implying that USP3's MDM2 deubiquitination is exclusive to hepatoma cells, promoting HepG2 cell proliferation. By deubiquitinating the RIG-I receptor, USP3 obstructs the type I interferon

pathway during the body's antiviral immune responses (Verrecchia and Rédini, 2018). In the absence of USP3 in the mouse model, increased levels of monoubiquitinated H2A and H2B were observed, along with a notable rise in spontaneous chromosomal breaks within the tissue cells. Conversely, there was a substantial reduction in the reserves of hematopoietic stem cells in vivo and a shortened lifespan of the mice (Gierisch et al., 2019). This highlights the crucial role of USP3 in maintaining the body's physiological equilibrium and stability during periods of the stress response. According to recent studies, the USP3 protein takes part in several physiological processes, including DNA damage and repair, control of the cell cycle, and proliferation of cells. Although the precise molecular pathways underlying the development and progression of disorders associated with USP3 remain incompletely understood, it is essential to deepen our understanding of USP3's molecular functions within cells and its connection with different diseases. Further research efforts may reveal a specific molecular target that could be crucial in diagnosing and treating clinical conditions.

5. USP role in osteoblastoma

Osteosarcoma is the leading primary bone tumor observed in children, adolescents, and young adults. Among adults, it ranks as the third most prevalent bone tumor. The global average annual incidence of osteosarcoma is reported to be 3.4 cases per million individuals. As the defining characteristics of this malignant bone tumor, an osteoid matrix, which, as the name implies, is the development of bone in the metaphysis and diaphysis region of long bones, specifically in the humerus, femur, and tibia is primarily responsible for this development (Gill and Gorlick, 2021). According to Liu et al. (2016), they observed an elevation of USP1 in individuals with OS in contrast to those without the condition. They also pointed out that the growth capability of U2OS, a cell line associated with OS, diminished when USP1 was suppressed. When USP1 is silenced in OS cells, it promotes osteogenic differentiation. Conversely, when USP1 is overexpressed in mesenchymal stem cells (MSCs), it prevents osteoblast differentiation and stabilizes IDs. It was shown that USP9x restricts the growth of OS cells by deubiquitinating SOX2 (Durer et al., n.d.). In U2OS cells, the deletion of USP39 cleaves poly-ADP ribose polymerase, causing cellular death, and stops mitosis during the G2/M phase in a p21-dependent manner. Furthermore, it has been established that the heightened presence of USP22 in osteosarcoma tissues contributes to the advancement of OS by impeding the PI3K/Akt signaling pathway. On the other hand, inhibiting USP22 was demonstrated to impede the spread, aggressiveness, and transformation of osteosarcoma cells from an epithelial to a mesenchymal state in laboratory settings. Additionally, it was found to halt the growth and spread of osteosarcoma in live animal models. Moreover, the observed increase in USP7 expression within osteosarcoma tissues has the potential to trigger the process of epithelial–mesenchymal transition (EMT) and initiate the activation of the Wnt/β-catenin pathway. As a result, these findings contribute to the progression and dissemination of osteosarcoma. Additionally, studies have indicated elevated levels of USP6, USP27x, USP41, and USP43 in HOS, U2OS, and MG63 cell lines associated with osteosarcoma. Conversely, it has been suggested that there exists an inverse correlation between the expression levels of USP6 and USP41 and the survival rates of osteosarcoma patients.

The progression of the tumor can be broadly segmented into two key phases: initial tumor expansion and the subsequent development of metastasis. Within this framework, several USPs have been recognized for their involvement in both phases of osteosarcoma tumor advancement. Concerning the proliferation of primary tumors in osteosarcoma, studies have indicated the involvement of USP1, USP22, and USP39 in regulating the growth of OS cells. Of the four USPs exhibiting elevated expression in OS cells, USP6 has been identified as capable of modulating cell proliferation. USP27x and USP41 have also been recognized for their roles in influencing cell proliferation within cancer cells. For instance, USP27 contributes to the regulation of primary liver tumor growth through the stabilization of cyclin E, whereas USP41 has a role in controlling the growth of lung cancer cells (Jemal, 2013).Therefore, it is possible to propose that USP27x, USP43, USP6, and through different pathways, may have the capacity to regulate the proliferation of OS cells and, consequently, the growth of primary tumors. Concerning the development of metastasis in OS, both USP1 and USP22 have been found to play roles in regulating the relocation and invasion of OS cells, consequently influencing the process of metastatic development. Of the four USPs that we found to be highly expressed in OS cells, USP43 and USP27x both have an impact on regulating EMT and, in turn, the progression of cancer across several subtypes. These findings indicate that in cases of OS with heightened USP43 expression, this protein might be involved in regulating EMT, which is a crucial cellular process linked to the progression of OS. The schematic diagram represents the USP's action on key functions in osteosarcoma progression (Fig. 10.2).

List of some USP interactions with target proteins associated with cell cycle progression is represented in Table 10.1.

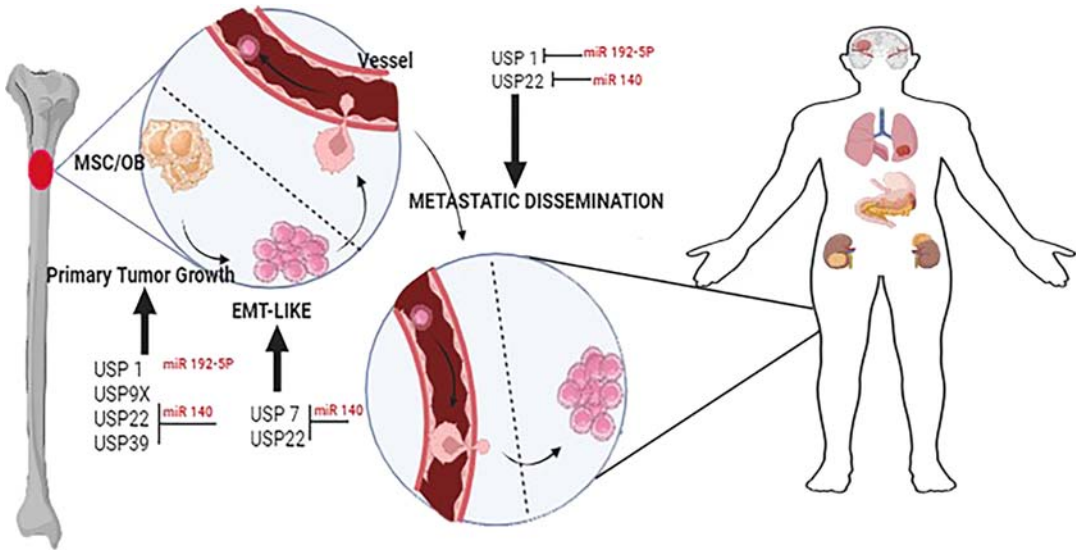

FIGURE 10.2 USP in osteosarcoma.

TABLE 10.1 List of ubiquitin-specific protease interactions with target proteins associated with cell cycle progression.

USPs	Targets	Model	Effects	References
USP2	Cyclin A1	T24 cells (bladder carcinoma)	USP2a overexpression induces cellular invasion and proliferation	Kim et al. (2012)
	Cyclin D1	HCT116 (colorectal carcinoma)	Knockdown of USP2a inhibits cellular growth by G1/S arrest	Shan et al. (2009)
USP3	CDC25A	HeLa cells (cervical adenocarcinoma)	USP3 knockdown provokes a delay in cell cycle progression and reduces the tumor growth in mice bearing tumor xenografts	Das et al. (2020)
	Klf5	HCC1937, HCC1806, and SUM149PT cells (breast carcinoma)	Knockdown of USP3 leads to a decrease in cellular proliferation and invasion	Wu et al. (2019)
USP7	PHF8 −cyclin A2	MCF7 (breast carcinoma)	Knockdown of USP7 reduces cellular proliferation, knockdown of USP7 reduces tumoral growth in mice bearing tumor xenografts	Wang et al. (2016)
	PLK1	DU145 and VCaP cells (prostate adenocarcinoma)	Knockdown of USP7 leads to a decrease in cellular proliferation and viability and an interruption of the G2/M cell cycle	Peng et al. (2019)
USP17	ELK1	HEK293T cells (kidney)	Knockdown of USP17 inhibits cellular proliferation and stops the G1/S cell cycle	Ducker et al. (2019)
	SET8−p21	MCF7 (breast carcinoma)	Knockdown of USP17 induces G1 phase arrest and apoptosis	Fukuura et al. (2019)
USP22	Cyclin B1	HCT116 cells (colorectal carcinoma)	USP22 knockdown prevents G2/M cell cycle progression and inhibits cellular proliferation, knockdown of USP22 decreases tumoral growth in mice bearing tumor xenografts	Lin et al. (2015)
	p15, p21, and cyclin D2	HEPG2 cells (hepatocellular carcinoma)	Knockdown of USP22 reduces cellular viability and promotes G0/G1 cell cycle arrest and apoptosis	Ling et al. (2012)
USP39	CDK1 and cyclin B1	HO-8910 and SKOV3 cells (ovarian carcinoma)	USP39 knockdown induces the arrest of the G2/M cell cycle and inhibits cellular proliferation	Yan et al. (2019)
		TT cells (thyroid carcinoma)	Knockdown of USP39 inhibits cellular proliferation and induces G2/M arrest	An et al. (2015)
	Unknown	SMMC-7721 cells (hepatocellular carcinoma)	USP39 knockdown inhibits cellular proliferation, stops G2/M cell cycle transition	Pan et al. (2015)
		SW1116 and HCT116 cells (colorectal carcinoma)	Knockdown of USP39 blocks cellular proliferation and G2/M phase	Xing et al. (2018)
USP42	Cyclin D1	AGS and MKN-45 cells (gastric adenocarcinoma)	Suppression of USP42 provokes G0/G1 cell cycle arrest and inhibits cellular proliferation	Hou et al. (2016)
	Cyclin E1	AGS cells (xenografts)	Knockdown of USP42 suppresses tumoral growth in mice bearing tumor xenografts	
USP50	Hsp90	U2OS (bone osteosarcoma)	Knockdown of USP50 blocks cells in G2/M	Aressy et al. (2010)

6. USPs and pediatric primary bone tumors

Among the various ubiquitin-specific proteases (USPs) studied in osteosarcoma (OS), USP1 has garnered the most attention. Liu and colleagues, through immunohistochemistry analysis of 30 OS patient biopsies, demonstrated elevated USP1 expression in tumor tissues compared with healthy tissues. Silencing USP1 in U2OS OS cells resulted in reduced proliferation and colony formation capacity in soft agar (Gaspar et al., 2018). The observed effects were attributed by the authors to a reduction in the protein levels of various factors that directly or indirectly regulate the proliferation of cells. These components, SIK2, MMP-2, GSK3β, Bcl-2, Stat3, cyclin E1 or A1, Notch1, and Wnt-1 are significant variables. USP1 plays a significant role in the response to cisplatin and contributes to the maintenance of the undifferentiated state of OS cells. Research indicates that USP1, in conjunction with DNA IDs, enhances its stability and promotes stem cell—like features in OS. Silencing USP1 in OS cells leads to the degradation of ID proteins and an increase in osteoblastic differentiation. In a study led by Zeng and Coll, it was discovered the OS tumor tissues expressed more USP7 than nearby healthy tissues. Interestingly, metastasis and advanced illness stages are linked to this elevated expression. OS cells lose some of their migratory and invasive potential when USP7 is inhibited. The impact of USP7 on the Wnt/β-catenin signaling system and its potential to modulate the epithelial-to-mesenchymal transition (EMT) are credited by the authors for these modifications. Zhang et al. (2017) and colleagues also showed a significant upregulation of USP22 expression in cell lines and OS tissues in another study. In vitro, USP22 silencing efficiently inhibits OS cell invasion, proliferation, and the transition from EMT. The authors attributed these effects to USP22's ability to alter the PI3K/Akt signaling pathway, indicating that USP22 could be a useful target for OS treatment. It was shown by Gan and associates that the USP39 deletion in U2OS cells interferes with the ability of the cells to proliferate as well as form colonies. These outcomes were connected to the activation of cell death, which was demonstrated by PARP cleavage in U2OS cells, and the arrest of OS cells in the G2/M phase of the cell cycle (Friebele et al., 2015).

In a recent research, Lavaud and colleagues found that the MG63, U2OS, and HOS cell lines have four overexpressed USPs: USP6, USP27x, USP41, and USP43. Notably, Kaplan-Meyer analysis revealed a correlation between patient survival and USP6 and USP41 expression. Moreover, preclinical in vivo investigations using an OS model demonstrated the pan-USP inhibitor PR619 efficiently inhibits the growth of primary OS tumors and the emergence of lung metastasis. Gierisch et al. (2019) and colleagues (2019) conducted another fascinating study that revealed USP19's critical function in controlling the stability of EWS-FLI1, the oncogenic fusion protein responsible for the development of Ewing sarcoma. Reduced EWS-FLI1 protein levels as a result of USP19 depletion lower protein activity.

7. Ewing's sarcoma

Ewing (1972) first identified on 14-year-old girl who was diagnosed with Ewing's sarcoma (ES), which is classified as "diffuse endothelioma of bone," a new kind of bone tumor. When it comes to primary malignant bone tumors, ES is the second most common kind, with an incidence rate of 1.5 occurrences per million following OS. This hasn't changed in decades.

ES is a type of cancer that most commonly affects people between the ages of 10 and 20. About half of all ES cases occur in people under the age of 15, and the other half occur in people more than 20 years old. At diagnosis, the median age is 15 years. ES is rarely seen in newborn babies. There are a few cases reported in the medical literature. Men are more likely to develop ES than women, with a ratio of three men to two women. ES exhibits a higher prevalence among individuals of Caucasian ethnicity compared with the African population (Gibson et al., 2018).

8. USP3 in esophageal squamous carcinoma

Esophageal squamous carcinoma (ESCC) is an exceedingly prevalent gastrointestinal cancer characterized by a significant burden of morbidity and mortality. Currently, it ranks as the ninth most common cancer worldwide in terms of incidence and the sixth leading cause of total mortality. ESCC primarily manifests as two main pathological subtypes: squamous cell carcinoma and adenocarcinoma. Diagnosis often occurs at an advanced stage due to the subtle nature of early ESCC symptoms, compounded by the absence of effective early detection methods. The chief contributors to mortality among ESCC patients are tumor invasion and metastasis. These processes hinge on the activation of proto-oncogenes, inactivation of tumor suppressor genes, and involvement of various pathways related to cell proliferation and apoptosis.

In 2021, Shi et al. (2021) and colleagues made an intriguing discovery regarding the interaction between USP3 and Aurora A. They observed that this interaction led to a decrease in the ubiquitination level of Aurora A, consequently inhibiting the proteasome degradation pathway linked to Aurora A. To delve deeper into this phenomenon, the researchers generated a deubiquitinated mimic of Aurora A, known as K143R. Remarkably, their experiments revealed that K143R significantly promoted the proliferation and invasion of ESCC cells, and this effect was not influenced by USP3's deubiquitination activity.

These findings collectively underscored the pivotal role of USP3's deubiquitination of Aurora A in instigating tumorigenic traits in ESCC. This study also revealed the versatility of the USP3 protein in orchestrating diverse cellular processes, including the proliferation of cells, the regulation of the cell cycle, and the repair of DNA damage, and shed light on the role of this protein. These processes are intricately tied to the initiation and progression of diseases (Aressy et al., 2010). Nevertheless, the exact molecular mechanisms governing the development and advancement of diseases associated with USP3 remain enigmatic. Consequently, there exists an urgent imperative for further investigations aimed at elucidating the molecular functions of USP3 in cells and its connections to various diseases. These research endeavors hold the potential to unveil molecular targets that could be invaluable for the diagnosis and treatment of clinical diseases.

8.1 Ubiquitination modifications in oral squamous cell carcinoma

Squamous cell carcinoma of the head and neck is one of the most prevalent forms of cancer (HNSCC) superfamily oral squamous cell carcinoma (OSCC). Because of significant genetic changes, OSCCs often have an aggressive phenotype and a higher propensity to show up

as distant and local metastatic lymph nodes. The development and progression of OSCC are influenced by etiopathogenetic factors such as tobacco use and deregulation. A study team used a combination of quantitative real-time polymerase chain reaction (qRT-PCR), immunohistochemistry, western blot protocol, and combined immunohistochemistry to assess its expression in several OSCCs (Xie and Xu, 2021). They discovered a correlation between the molecule's significant overexpression and induced migration, invasion, and proliferation. The protease 14 (USP14) specific to ubiquitin is another molecule implicated in the progression of OSCC. A research team found a notable overexpression of the marker in OSCC cell cultures and tissue specimens using experimental colony formation analysis. It is interesting to note that a poor prognosis was linked to USP14 upregulation. Because of these factors, the authors proposed that USP14 is a molecule that is necessary for focused treatment approaches. Alongside the overactivation of DUB3, the development and progression of OSCC are facilitated by the elevated expression of ubiquitin-specific protease 22 (USP22), a deubiquitinating hydrolase. When USP22, survivin, and Aurora B molecules were coanalyzed, a research team found that all of them had high expression levels. It is interesting to note that in a molecular study, when RING finger protein 139 (RNF139) E3 ubiquitin ligase was activated, the growth of malignant cells was reduced. Ubiquitination is crucial for controlling protein metabolism because it degrades proteins and maintains intracellular homeostasis. Many molecules are involved in the signaling pathways for ubiquitination and sumoylation. Targeting and identifying a wide range of protein substrates is the Ub-PS. Every substrate protein is altered by ubiquitin conjugation, which controls the protein's biochemical destiny (degradation). Regulation of the autophagy mechanism is one of Ub-PS's primary functions. Remarkably, Ub-PS stimulates bulk autophagy at every stage initiation, execution, and termination. Ub-PS and autophagy interact in the cell microenvironment. Additionally, Ub-PS is connected to the DNA damage response and oxidative cellular stress. It is interesting to note how important Ub-PS's functional role in controlling ribosome machinery appears to be. Carcinogenesis affects the respective MEEK/MAPK/ERK, TGF-B, and JNK-dependent signaling pathways directly, which contributes to the development and progression of malignant diseases (Kyrodimos et al., 2022).

9. Conclusion

Ubiquitin protease kinase 3 plays a crucial role in the development and progression of various cancers, including bone cancer and squamous cell carcinoma. Elevated UPK3 expression increases tumor cell proliferation, invasion, and metastasis. UPK3 achieves this by deubiquitinating and stabilizing oncoproteins such as c-Myc and β-catenin, inhibiting tumor suppressors such as p53 and PTEN, and facilitating EMT. As a promising therapeutic target, UPK3 inhibitors such as ML340 and PIK-75 have shown promise in preclinical studies, inhibiting the growth and metastasis of cancer cells. Clinical trials are underway to evaluate the safety and efficacy of UPK3 inhibitors in treating bone cancer and SCC, with their outcomes expected to determine the potential for UPK3-targeted therapies in these cancers. Overall, UPK3 appears to be a promising therapeutic focus for the treatment of bone cancer and SCC.

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CHAPTER

11

Serine protease: Structure, classification, mechanism and role in breast cancer

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1. Introduction

Proteases in general are known as proteinases, peptidases, or proteolytic enzymes that catalyze the hydrolysis of peptide bonds, which links amino acids in the protein and polypeptide (Poddar et al., 2017). Proteases are distributed in intracellular (found in organelles such as cytosol, mitochondria, endoplasmic reticulum) or extracellular spaces (gastrointestinal tracts) bearing a molecular size ranging from ~ 20 kDa to 0.7–6 MDa (Bertenshaw et al., 2003). Altogether 2%–4% of genes in a genome encode for proteolytic enzymes. The entire complement of peptidases present within the genome is termed as degradome. Proteases are divided into exo (cleave at N-terminal) and endopeptidases (cleave at C-terminal), serine proteases, threonine, aspartic, glutamic acid, cysteine proteases, and metalloproteases (based on nature of functional groups at active site) (Rao et al., 1998). Proteases are also classified as acidic, alkaline, and neutral based on the optimal pH. The source of these proteases includes plants (for example, papain, bromelain, keratinases), animals (trypsin, chymotrypsin, pepsin, rennin), and microbes (from bacteria, fungus, virus that are either acidic, alkaline, or neutral proteases). MEROPS (https://www.ebi.ac.uk/merops/) is a database of proteases developed by Barret and team, which gives the detailed information on classification, substrates and inhibitors of proteases (Rao et al., 1998; Page and Di Cera, 2008). The four peptidase families account for over 40% of human degradome, which includes the ubiquitin-specific peptidase (clan CA family c19), Zn-dependent adamalysins (clan MA family M12 subfamily B), prolyl oligopeptidases (clan SC family S9), and the trypsin-like serine peptidases (clan PA family S1 and subfamily A), which forms the largest group in the human genome (Page and Di Cera, 2008).

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Proteases majorly regulate the fate, localization, and activity of many proteins, their interactions, and regulation of molecular signals (Zamyatnin and Parodi, 2022). Proteases also influence the events from DNA replication to cell differentiation. Alteration in proteolytic systems underlies multiple pathological conditions such as cancer, neurodegenerative disorders, and inflammatory and cardiovascular diseases. Accordingly, because of their biological importance, and apart from inducing the tumorigenesis, they also play an important role in signaling pathways or tumor suppression. Hence, many proteases have now become the major focus of attention for the pharmaceutical industry as potential drug targets or as diagnostic and prognostic biomarkers in cancer (Dudani et al., 2018; Zamyatnin and Parodi, 2022).

Cancer is the condition of cells, where the cells grow and proliferate in an uncontrolled manner rather than responding appropriately to the signals that control normal cell behavior. According to Siegel et al. (2021), cancer accounts for 18% of all deaths and remained the second leading cause of death after heart diseases. The role of proteases in cancer can be traced back to 1946, and the role of specific and individual proteases began in 1970s (López-Otín and Matrisian, 2007). This knowledge got wider response when Fisher first proposed the tumor associated proteolytic activity of degradation of cell matrix and invasion to the surrounding normal tissues. In the next decades, the prometastatic activities and role in cellular invasion via gain of function or loss of function associated with secreted members of serine, cysteine, and metalloproteases were studied through molecular biology studies. The recent findings revealed the involvement of proteolytic enzymes at every stage of tumor progression, including proliferation, adhesion, migration, differentiation, angiogenesis, senescence, autophagy, apoptosis, and evasion of the immune system. To date, there are around 176 serine proteases among the 569 human proteases in total (López-Otín and Matrisian, 2007). Moreover, several serine proteases are associated with dual functions in cancer such as cancer progression and protection from cancer in specific instances. Serine proteases such as kallikreins, testisin, and prostasin and dipeptidyl peptidase 4 act as antitumor proteases in testicular, prostate cancer, tumor of melanocytic cells, ovarian cancer by activating the antimitotic pathways, inhibiting the actin polymerization and altering the adhesion properties (Martin and List, 2019). Wherein, an increased expression of kallikreins (KLK1-KLK15), hepsin and urokinase, matriptase, trypsin, granzymes, and thrombin are associated with induction of cancer including hormonally regulated carcinomas, prostate, lung, gastric cancer, squamous cell carcinoma (SCC), tongue, lip, larynx, gingiva, and colorectal and breast cancer cells (Cullen et al., 2010; Filippou et al., 2016; Henneke et al., 2010; Martin and List, 2019; Rousalova and Krepela, 2010; Soreide et al., 2006; Yamamoto et al., 2003; Zhao et al., 2020).

Breast cancer is the most commonly diagnosed cancer in women accounting for more than 1 in 10 new cancers diagnosed each year (Siegel et al., 2021). Invasive ductal carcinoma is the most common type of breast cancer accounting for about 70% of tumors, and about 15%–20% of tumors are invasive lobular carcinomas. It is the second leading cause of death among women aged between 20 and 49 years (Alkabban and Ferguson, 2023). Breast cancer evolves silently and is usually discovered during the routine screening. Formation of breast lumps, change of breast shape or size, and nipple discharge are the indications of breast cancer by physical examination (Alkabban and Ferguson, 2023). Imaging such as mammography, magnetic resonance imaging (MRI), ultrasound, computerized tomography, positron emission tomography (PET), and tissue biopsy help to diagnose breast cancer (Wang, 2017). Assessment of the estrogen receptors (ERs), progesterone receptors (PRs),

and human epidermal growth factor receptor 2 (HER2), expression status of tumors has been used in clinical decision-making for many years (Weiderpass and Stewart, 2020). If unexamined, tumor tends to spread lymphatically and hematologically, leading to metastasis and poor diagnosis (Wang, 2017). The treatment modality includes lumpectomy or removal of tumor or small margin of surrounding healthy tissues; mastectomy is the removal of entire breast tissues, sentinel node biopsy (removal of a limited number of lymph nodes), axillary lymph node biopsy (removal of several lymph nodes), radiation therapy, chemotherapy, hormone therapy, targeted therapy, immunotherapy, and palliative care (Hortobagyi, 1998; Tong et al., 2018).

Despite having various treatment modalities for breast cancer, they are associated with certain limitations. Mammography is less effective if subjective under 40 years old or dense breast, it is less sensitive to small tumors and does not provide eventual disease outcomes (Hortobagyi, 1998; Tong et al., 2018). Other treatment including MRI is expensive and low specificity, leading to overdiagnosis (Wang, 2017). The nonspecificity, dilution, and degradation of analytes are the limitations associated with the detection of cancers using blood biomarkers (Dudani et al., 2018). Also, the incomplete understanding of genotype/phenotype correlation limits the broad application of genetic variants as biomarkers for precision medicine. Recent research is more focused on proteases that play critical roles in almost every hallmark of cancer, and the numerous processes regulated by the proteases are broadly dysregulated and functionally distinct in tumors (Dudani et al., 2018; Zamyatnin and Parodi, 2022).

2. Serine proteases—structure and classification

The serine proteases that compose one-third of all proteases (around 33% according to the MEROPS database) are structurally beta proteins, and core structure is composed of two similar domains namely the N and C domains (Chen et al., 2013; Pham, 2008; Wiedow and Meyer-Hoffert, 2005). The three major residues His57, Asp102, and Ser195 known as catalytic triads are present in the catalytic center of the serine protease (Fig. 11.1). The Asp102 and His57 are at the N -terminal domain whose function is to cooperate with C-terminal domain in maintaining the structural stability (Poddar et al., 2017). The Ser195 present in the C-terminal domain performs the main catalytic function of the serine proteases. Further, oxygen-ion pores (for stabilizing the catalytic intermediates), substrate-specific pockets, backbone substrate binding site, and two important conserved disulfide bonds are also located on the C-terminal domain (Fig. 11.1).

Over 26,000 serine proteases are grouped into 13 clans and 40 families (Fig. 11.2) (Page and Di Cera, 2008). Distribution of each clan across the species varies, where clan PA peptidase are highly represented in eukaryotes. The clan PA consists of family S1 that is further divided into subfamilies S1A and S1B. S1A is involved in extracellular processes, whereas S1B is involved in the intracellular protein turnover. Of 699 peptidases in man, 178 are serine peptidases and 138 of them belong to the S1 peptidase family of clan PA. The clan PA bearing the chymotrypsin fold is the largest group of serine peptidases; S1A peptidases form the most part in the human genome, which is grouped into six functional categories including digestion, coagulation, immunity, tryptase, matriptase, and kallikrein. The trypsin-like serine

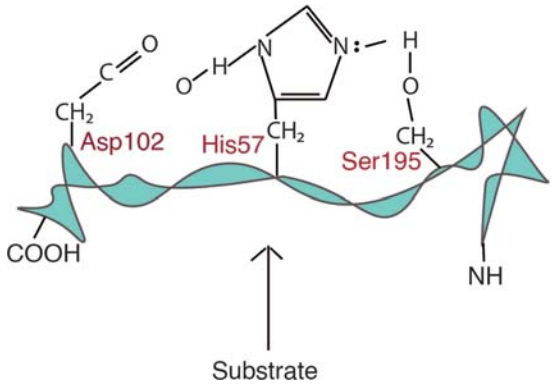

FIGURE 11.1 Structure of serine proteases.

peptidases (clan PA family S1 and subfamily A) are the largest group of homologous peptidases in human genome that includes trypsin, neurotrypsin and neurospin (Almonte and Sweatt, 2011). The other class of serine peptidase includes the thrombin-like protease namely, thrombin, tissue plasminogen activator (tPA), and plasmin (Almonte and Sweatt, 2011). Some of the serine proteases are anchored to the membrane such as type 1 transmembrane tryptase γ1 (also known as PRSS31) and type 2 transmembrane tryptases (TTSPs) that belong to the S1 family of serine proteases that include prototypic chymotrypsin and trypsin. There are several TTSPs including matriptase, matriptase-2, hepsin, TMPRSS2, TMPRSS3, TMPRSS4, and TMPRSS13, prostasin (PRSS8), and testisin (PRSS21) expressed differentially in cancer types (Martin and List, 2019). Kallikrein (KLKs) family of clan PA of serine proteases includes 15 members (KLK 1−15) (Filippou et al., 2016; Kalinska et al., 2016) (Fig. 11.2).

Clan SB peptidases are most prevalently seen in plants and bacterial genome. Clan SC peptidases are the second largest family of serine protease in human genome. S9 is the largest family of clan SC peptidases with 14 homologs present in human genome. Prolyl oligopeptidases (POP) and dipeptidyl peptidase IV (DPP-IV) are best characterized. Acetylcholinesterases (ACEs) are the nonproteolytic members of S9 family of serine proteases. Clan SE peptidases are majorly found in bacteria with minimal distribution in higher organisms. However, LACT-1 peptidases are the only clan found in human genome. The clan SF peptidases are found in eukaryotes where five representatives of S24 family are represented in the human genome. The human genome contains two clan SJ peptidases and three clan SK peptidases. The clan SH, SP, SQ, SR, and SS are populated by a limited number of peptidases. Clan ST is involved in Alzheimer's disease.

3. Mechanism of action

Serine proteases are synthesized in an inactive zymogen form that requires posttranslational modification for their activation. The activation of serine proteases is based on charge relay system, which is an irreversible process whose activity is regulated at three different stages. Hence, the first step involves expression of serine proteases, and the second step

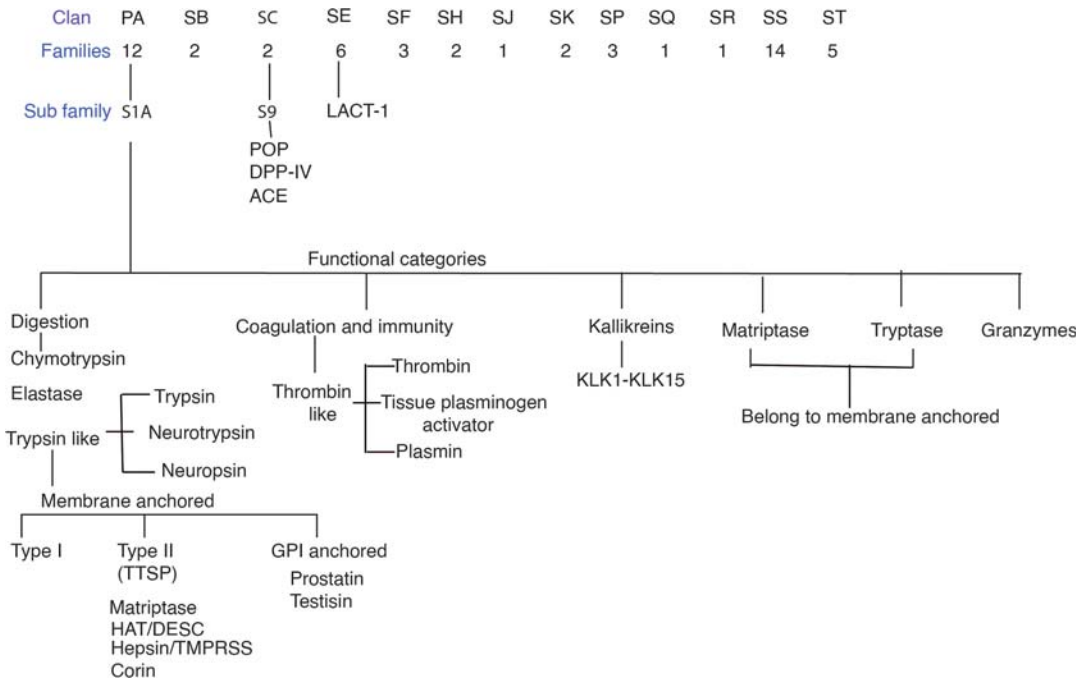

FIGURE 11.2 Classification of serine proteases.

involves posttranslational activation by other proteases to remove N-and/or C-terminal signaling peptides present in the serine protease structures. The final third step, inactivation of enzymes takes place by their inhibitors namely serpins (serine protease inhibitors).

During their mode of action (Fig. 11.3), peptide binds to the active site of the enzymes in such a way that the carbonyl part of the peptide is present close to the nucleophilic serine (Poddar et al., 2017). The serine residue of the catalytic triad is activated by the electron-rich nitrogen atom of the histidine residue. The serine OH attacks the carbonyl −C of peptides to generate a tetrahedral intermediate, where a new covalent bond is formed between the carbonyl −C of the peptide and −O atom of the serine and, between the H-atom of serine and N-atom of histidine. Due to the positive charge generated on the histidine, it donates the H-atom to the N-atom of the sessile bond of the peptide. Hence, the sessile bond is broken in which sessile bond is now covalently bond with −H atom of histidine. The negative charge on the oxygen atom that was formed previously on the −C═O moves back to recreate a double bond. The peptide bond breakage results in the release of N-terminus part of the peptide, and C-terminus part of the peptide is covalently attached to the serine residue, generating an acyl−enzyme intermediate. The histidine residue of the catalytic triad is back into the original form as an acid catalyst (Fig. 11.3).

During the time of enzyme regeneration, the water molecule plays a significant role in the process of catalysis reaction. The electron-rich nitrogen atom of the histidine residue extracts the proton group of water molecule. The OH part of the water molecule now acts as nucleophile and attacks the electron sink of −C═O part of the peptide. Now the new N−H bond is

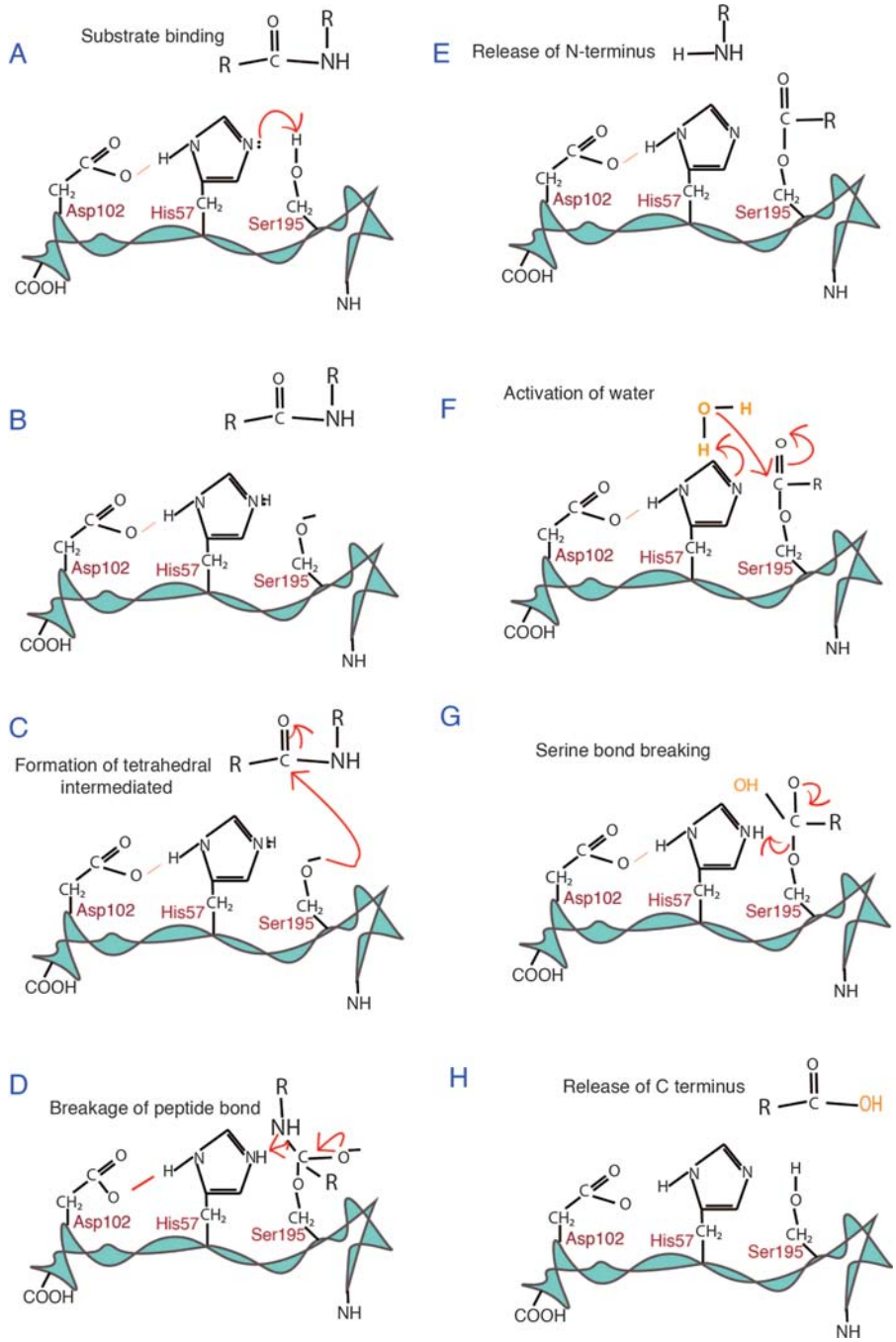

FIGURE 11.3 Mode of action of serine proteases.

formed like before where histidine residue again carries a positive charge, which is stabilized by the H-bond of aspartic acid of the catalytic triad. The electron pairs from the −C═O of the substrate move back to the oxygen atom, and the bond between the −OH of the water and the C- of the substrate is formed generating a tetrahedra intermediate. To neutralize the positive charge generated on histidine, the breakage of -N-H of histidine takes place resulting in a new covalent bond between the −H atom of N−H bond of histidine and −O atom of the serine residue by breaking the bond between the carbonyl −C of the peptide and O atom of the serine. The C-terminus of the peptide is released with −H group of water to regenerate the enzyme. Hence, the peptide is hydrolyzed with the help of proteases by adding the −H atom to the N-terminus and −OH to C- terminus of the peptide bond of the substrates. Dysregulation at any point of these stages leads to their uncontrolled activity and undesirable hydrolysis of peptide bonds that are associated with disease such as chronic obstructive pulmonary disease (COPD), cancer progression, and metastasis.

4. Physiological function of serine proteases

Serine proteases are responsible for coordinating various physiological functions, including digestion, immune response, blood coagulation, reproduction, and the complement system (Neitzel, 2010; Vizovisek et al., 2021).

4.1 Digestion

Trypsin, chymotrypsin, and elastases are the serine protease found in the digestive system of vertebrates, which cleave specific amino acids in the polypeptide chains (Poddar et al., 2017). For example, trypsin prefers to cleave the peptide bonds that have lysine and arginine, chymotrypsin prefers aromatic amino acids (phenyl alanine, tyrosine and tryptophan), and elastase prefers to cleave the amino acids with short side chain groups such as alanine in their cleavage sites.

4.2 Immune response

Tryptases are the major components in the secretory granules of the mast cells that mediate proinflammatory signaling through protease-activated receptor (PAR), whereas some of the serine proteases are also found in the granules of cytotoxic T lymphocytes, natural killer cells, dendritic cells, B cells, and mast cells, which are commonly called as granzymes (Patel, 2017). The urokinase plasminogen activator (uPA) participate in the physiological functions of cell growth to tissue remodeling of immune system and inflammation, fibrinolysis, embryogenesis, angiogenesis, cell migration and activation, and differentiation of white blood cells (Heutinck et al., 2010).

4.3 Blood coagulation

The plasminogen activator (PA) system comprises two serine proteases uPA and tissue plasminogen activator (tPA), which catalyze the formation of active protease plasmin from their inactive zymogen called plasminogen that breaks the extracellular proteins (Heutinck et al., 2010; Kini, 2006). The tPA acts via fibrin-dependent pathway for blood clot dissolution process, while the uPA performs through fibrin independent pathway and largely acts on the cell surface receptor-bound plasminogen activator such as uPAR, which controls the pericellular proteolysis of the system, and involves the degradation of ECM resulting in the invasion and metastasis of cancer. Plasmin and thrombin are the serine proteases found active in the coagulation cascade and complement pathway. During the blood clotting, approximately 20 soluble plasma factors, including fibrinogen (FI), prothrombin (FII), thromboplastin (FIII), and calcium ions (FIV), which are the precursors of proteolytic enzymes (known as zymogens), circulate in an inactive form in the blood stream, which will cleave the downstream proteins to activate thrombin or fibrin, leading to blood clotting or coagulation, respectively.

4.4 Reproduction

Proteolytic processing events are necessary in reproductive processes including gametogenesis, fertilization, and embryonic development (Kiyozumi and Ikawa, 2022). Acrosin, a serine protease, is a major component of acrosome. The fertility of the humans is correlated with several serine proteases including plasmin, matriptase, nexin-1, and testisin.

4.5 Protein degradation and signaling

Aberrant protease expression contributes to various hallmarks of cancer, viz, uncontrolled growth, survival of cells by escaping the death signal, angiogenesis, invasion and metastasis, and immune system evasion (Rakash, 2012). Proteases including kallikreins are the secreted serine proteases found in the human pancreas, where human tissue kallikreins (hKs) convert the high-molecular-weight proteins into peptides known as kinins (Khatab et al., 2023). Human plasma kallikreins cleave the high molecular kininogens into bradykinin, a potent vasodilator nonapeptide. There are 15 tissue kallikrein genes reported named as KLK1 to KLK15 that are regulated by the steroid hormones. These are responsible for the ECM degradation and function in the metastasis of breast, lung, ovarian, testis, and prostate cancer (Khosroabadi and Yousefnia, 2023; Stuardo-Parada et al., 2023). Kallikreins are found to be deregulated even in lung adenocarcinoma, pancreatic, and lymphoblastic leukemia. Matriptases are the membrane-bound S1 peptidases similar to trypsin where the high gene expression level for matriptases is associated with a variety of cancer types (Kim, 2023). Therefore, the altered function and expression pattern of serine proteases are correlated with many diseases including arthritis, emphysema, and cancer.

5. Serine proteases as diagnostic and prognostic markers in breast cancer

The differential expression of serine proteases accompanied by either increased or decreased enzymatic activity is linked in breast cancer condition. Visualization of aberrant activities of these enzymes during the pathological conditions in biological samples either in vivo or ex vivo is very often crucial for understanding their role in health and disease (Garland et al., 2016). Breast cancer progression is due to the expression of extracellular or cell surface proteases that cleave the extracellular matrices and activate the downstream signaling molecules responsible for the invasion and metastasis of the cancer cells (Martin and List, 2019; Poddar et al., 2017). The current diagnostic includes imaging of individual enzymes including proteases, which rely on the use of small molecule chemical probes that have gained importance in both basic research and translational medicine (Scott et al., 2021). These compounds because of their strong infrared fluorescence emission or radioactivity penetrate deep into the skin and enable the visualization of noninvasive proteases. These techniques provide accurate information on the activities of the targeted proteases at different stages during the diagnosis. The proteases in serum and plasma specimens can be detected by immunological methods, activity-based assays, optical, or MS monitoring. Bioluminescence resonance energy transfer (BRET)−based quantum dot (QD) nanosensors detect the activities of proteases, which is a highly sensitive detection method of proteases (Yao et al., 2007). The monoclonal antibodies (mAbs) are also used to distinguish the closely related protease family members (Lopez et al., 2019). Due to their stability in the serum, potential to cross the blood−brain barrier and improved effector functions offer significant advantages over small-molecule therapeutics. Hence the specific inhibitory mAbs targeting individual proteases are of biomedical importance.

The well-known tumor-associated proteases such as urokinase plasminogen activator (uPA) can be identified by activity-based proteomic profiling (ABP) (Suaifan et al., 2016). ABP is a method that uses site-directed chemical probes that irreversibly bind to the active proteases in biological samples but not into inactive zymogens. The specific proteases reflect the biological phenotype and metastatic potential of the tumor tissues. The prognostic relevance of uPA for breast cancer has been demonstrated in numerous clinical studies and has reached the highest level of evidence (Suaifan et al., 2016). The serine protease HTRA1 (Prss11), which belongs to the class of high temperature requirement proteins (HTRA), is thought to act as a tumor suppressor due to its ability to attenuate cell motility, growth, and invasiveness under the normal physiological condition (Chien et al., 2009). But during the early stages of breast cancer, the CpG-hypermethylation leads to the downregulation of HTR1, thereby causing the migration and invasion of cancer cells (Lehner et al., 2013). The lower expression of HtrA1 is significantly related to the poor prognosis of breast cancer, whereas the HtrA2 regulates the CCR2-mediated breast cancer cell growth and cellular invasion (Wu et al., 2021).

Similarly, the subfamily members of type 2 transmembrane serine proteases (TTSPs) degrade the components of extracellular matrix and play a significant role in the tumor growth, invasion, and metastasis (Netzel-Arnett et al., 2006). Humans express 17 TTSPs, which are divided into four different subfamilies viz., hepsin/transmembrane protease, serine (TMPRSS), matriptase, human airway trypsin-like (HAT)/differentially expressed in squamous cell

carcinoma (DESC), and the corin subfamilies (Kim, 2023). Increased expression of these proteins is associated with a poor prognosis. Normal physiological conditions maintain a homeostatic ratio between the proteases and their endogenous inhibitors, whereas during the cancerous condition, the ratio is imbalanced. The overexpression of TMPRSS1, TMPRSS4, TMPRSS6, TMPRSS7, TMPRSS11E/DESC1, and TMPRSS13 is associated with breast cancer condition (Li et al., 2017; Luostari et al., 2014). DESC1 converts the pro-urokinase-type plasminogen activator (uPA, PLAU) to active uPA, suggesting to be a suitable breast cancer biomarker with appropriate adjuvant therapy (Luostari et al., 2014). The PRSS23 is coexpressed with the estrogen α, which becomes the prominent biomarker and therapeutic target for breast cancer (Chan et al., 2012). Matriptases are widely expressed in tissues rich in epithelial cells including breast and tumors of epithelial cells originated from these tissues (Al-Awadhi and Luesch, 2020). The recent research also identified the role of matriptase in inflammatory breast cancer, which is rare and aggressive form of invasive breast cancer (Tanabe and List, 2017). It is interesting to know that the genetic variants in these genes alter their function, leading to the cancer onset and progression, thereby affecting the patient outcome. Luostari et al. (2014) estimated the association of genotypes against breast cancer risk and breast cancer-specific survival. Their results demonstrated the combined effect of variants in TTSPs and their related genes in breast cancer risk and patient outcomes. However, functional analysis of these variants will give a clear understanding of these gene families that may further improve individualized risk estimation and the development of new strategies for breast cancer. The other group of serine proteases includes the kallikrein family of serine proteases, where the human tissue KLKs are encoded by 15 genes (hKLK1-15) and expressed differentially in the tissues, fluids, and organs (Suaifan et al., 2016). Research has investigated the potential values of hKLKs as prognostic and diagnostic biomarkers in breast cancer. An eightfold increased expression of hKLK4 was observed in the breast cancer conditions; also, a positive expression of hKLK12 was observed in the triple-negative breast cancer patients (Mangé et al., 2008). The hKLK3 showed a tumor suppressive action and negatively regulated the hormone-dependent breast carcinoma, whereas hKLK5 and -7 were indicators of unfavorable prognosis in breast cancer. The downregulation of KLK10 in breast cancer acts as a predictive biomarker for response to tamoxifen therapy. KLK14 expression is more frequently present in patients with advanced stages and may be applicable as diagnostic/prognostic breast cancer biomarker. However, due to their substrate specificities, targeted KLK inhibitors can be selectively detected and developed. Hence, the proteomic analysis of the body fluids reveals the role of proteases as diagnostic/prognostic biomarkers. The molecular profiling identifies some of the KLKs, TMPRSS as prognostic biomarkers as well as therapeutic targets of breast cancer. Also, the elevation of certain proteases in breast cancer drives the need for screening potent and selective protease inhibitors as a novel therapeutic anticancer agent. Hence, optimization of detection methods capable of detecting conventional genetic and proteases as a biomarker would provide optimal panel toward breast cancer detection, diagnosis, monitoring of progression, and therapeutic success.

6. Conclusion and future perspectives

Serine proteases, the major class of proteases, are the key regulators of various physiological processes including digestion, immune response, blood coagulation, reproduction, and

the complement system. The kallikreins, granzymes, glycosyl phosphatidyl inositol-anchored (GPI-anchored) serine proteases, matriptases, and hepsin are some of the serine proteases whose activity if gets dysregulated leads to pathological conditions such as cancer growth, tumor metastasis, inflammation, cardiovascular, and autoimmune disease. Hence, these molecules are recognized as important and tractable drug targets. The differential expression of serine proteases in disease and its progression will be important in discovering biomarkers and therapeutic targets. Recent research focuses on activity-based probes such as FP-biotin that give idea on the activity of the proteases rather than on expression levels. These aspects are useful since the proteases are synthesized in an inactive form called zymogens and activated later to perform their function. Also, the use of other techniques such as cDNA microarray technology, 2D-gel electrophoresis or development of specific antibodies that can specifically target the specific proteases help to study the expression patterns and investigate functions of proteases.

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SECTION II

Proteases as diagnostics and prognostics biomarkers

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CHAPTER

12

Protease activator receptor-1 and matrix metalloproteinase-1 as prognostic factors and a novel therapeutic targets for gastric cancer

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1. Introduction

Proteases remain a group of enzymes that play serious parts in various physiological processes, including tissue remodeling, immune response modulation, and cell signaling (Overall and Blobel, 2007). In the context of cancer, these enzymes have gained substantial attention due to their involvement in cancer growth, metastasis, and invasion (Duffy et al., 2000). Proteases contribute to cancer progression by facilitating several critical steps of the metastatic cascade, such as degradation of the extracellular matrix component (ECM), promotion of angiogenesis, and modulation of immune responses (Egeblad and Werb, 2002; Stamenkovic, 2000). Because of their capacity to cleave ECM components, cancer cells can infiltrate nearby tissues and enter blood vessels (Liotta et al., 1991). This phenomenon highlights their role in shaping the tumor microenvironment and promoting cancer spread (Fingleton, 2006). The dysregulation of proteases in cancer has prompted the development of various therapeutic strategies to target these enzymes and their associated pathways. Inhibitors targeting specific proteases have shown promise in preclinical studies and clinical trials,

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with some already approved for cancer treatment (Turk et al., 2000). The challenge lies in achieving specificity while avoiding off-target effects on normal physiological processes (Egeblad et al., 2010). Among the numerous proteases implicated in cancer, protease activator receptor 1 (PAR-1) and matrix metalloproteinase 1 (MMP1) have gained attention in the context of gastric cancer. G protein–coupled receptor PAR-1 has been connected to angiogenesis, migration, and proliferation of cancer cells (Camerer et al., 2010). MMP1, an associate of the matrix metalloproteinase family, is related to ECM degradation and cancer cell attack (Kessenbrock et al., 2010).

2. Gastric cancer: Pathogenesis and clinical relevance

2.1 Gastric cancer: An overview of etiology and molecular mechanisms

The multifactorial etiology of gastric cancer (GC) includes genetic, environmental, and lifestyle factors. GC is a complex disease (Crew and Neugut, 2006). One major risk factor is a persistent infection with the stomach lining-colonizing bacteria *Helicobacter pylori* (Correa and Houghton, 2007). *H. pylori* colonization leads to chronic gastritis, progressing to atrophic gastritis; *H. pylori's* role in inducing genetic and epigenetic alterations within the gastric epithelium contributes to its carcinogenic potential (Amieva and Peek, 2016). Genetic factors play a significant role in gastric cancer susceptibility. CDH1 mutations are associated with hereditary diffuse gastric cancer (HDGC), an autosomal dominant syndrome (Hansford et al., 2015). There is also evidence linking the risk of gastric cancer to additional genetic variations, such as those in genes that encode cytokines and their receptors (El-Omar et al., 2000). Fig. 12.1 illustrates the staging of gastric cancer.

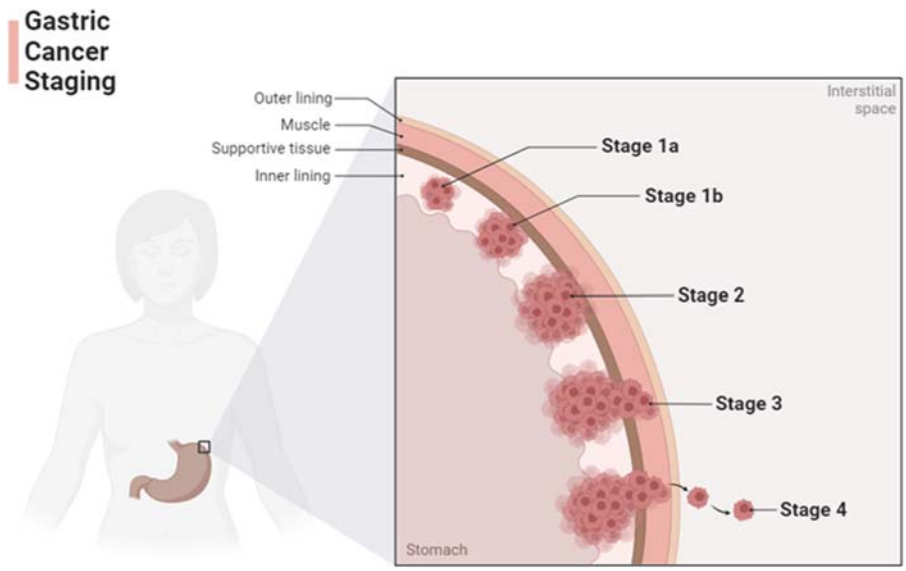

FIGURE 12.1 Stages of gastric cancer.

Stage 0 (carcinoma in-situ): Cancer cells limited to stomach lining; Stage I (Localized Invasion): Tumor extends into stomach wall; Stage II (advanced local invasion): Deeper tumor invasion, possible lymph node involvement; Stage III (regional spread): Extensive tumor invasion, multiple lymph node involvement; Stage IV (distant metastasis): Cancer spreads to distant organs or tissues.

Molecular mechanisms driving gastric cancer include alterations in oncogenes (e.g., HER2) and tumor suppressor genes (e.g., TP53), as well as dysregulation of signaling pathways such as Wnt and PI3K-AKT (Polk and Peek, 2010; Correa, 1986). Amplification and overexpression of the HER2 gene are observed in a subset of gastric cancers, leading to increased proliferation and survival signals (Gravalos and Jimeno, 2008). Mutations in the TP53 gene, a well-known tumor suppressor, are prevalent in gastric cancer, contributing to genomic instability and evasion of growth control (Corso et al., 2011). Signaling pathways also play an important role in gastric cancer progression. The beginning of the Wnt pathway due to mutations in genes such as CTNNB1 contributes to uncontrolled proliferation (Cristescu et al., 2015). Dysregulation of the PI3K-AKT pathway promotes cell survival, growth, and resistance to apoptosis (Engelman and Cantley, 2008). GC frequently manifests slowly, which increases mortality and presents a difficult prognosis (Iwu and Iwu-Jaja, 2023). Treatment approaches include surgery, chemotherapy, targeted therapies, and immunotherapy (Ajani et al., 2016). However, the heterogeneity of gastric cancer poses significant treatment challenges, necessitating personalized treatment strategies (Bang et al., 2010).

2.2 Emerging biomarkers in gastric cancer diagnosis and treatment

Promising biomarkers for gastric cancer diagnosis and treatment guidance have been identified thanks to advancements in molecular profiling (Cristescu et al., 2015). These include HER2 amplification, PD-L1 expression, and microsatellite instability (MSI) (Fig. 12.2) (Bang et al., 2015; Le et al., 2017). These biomarkers offer potential for targeted therapies and immunotherapy, transforming the landscape of gastric cancer treatment.

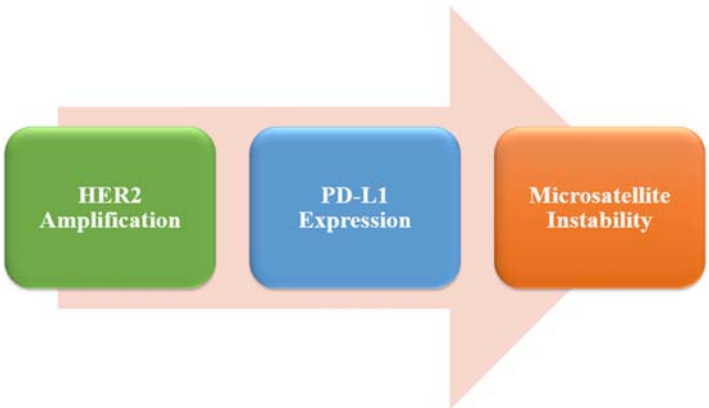

FIGURE 12.2 Treatment steps for gastric cancer.

Emerging biomarkers in gastric cancer: Molecular profiling advancements have unveiled potential diagnostic and treatment-guiding markers, such as HER2 amplification, PD-L1 expression, and microsatellite instability (MSI).

2.2.1 HER2 amplification

The well-known breast cancer biomarker of human epidermal growth factor receptor 2 (HER2) amplification has piqued interest in stomach cancer as well. A poor prognosis and aggressive tumor behavior are linked to HER2 overexpression. Trastuzumab, an anti-HER2 monoclonal antibody, has shown efficacy in HER2-positive advanced gastric cancer patients, leading to improved outcomes (Bang et al., 2010).

2.2.2 PD-L1 expression

Programmed death-ligand 1 (PD-L1) is a barrier protein that interrelates with its receptor PD-1 on immune cells to suppress the immune response when it is overexpressed in tumors. It has been noted that stomach cancer expresses PD-L1, and immune checkpoint inhibitors that board the PD-1/PD-L1 pathway have demonstrated promise in human trials (Fuchs et al., 2018).

2.2.3 Microsatellite instability

Short, repetitive DNA sequences that are prone to mistakes during DNA replication are known as microsatellites. Microsatellite instability (MSI) is a biomarker of deficient DNA mismatch repair (dMMR) machinery, resulting in a hypermutated phenotype. MSI-high tumors exhibit increased immunogenicity and are more responsive to immune checkpoint inhibitors (Le et al., 2017). These emerging biomarkers offer new avenues for targeted therapies and immunotherapies, ushering in a new era of precision medicine for gastric cancer patients. Further research and clinical trials are essential to fully understand their implications and possible benefits in the setting of gastric cancer treatment.

3. Protease activator receptor 1 in gastric cancer

The G protein−coupled receptor family's protease activator receptor 1 (PAR-1) has drawn notice for its significant part in the growth of gastric cancer. PAR-1 is activated by proteases such as thrombin and other serine proteases, leading to intracellular signaling pathways that influence various cellular processes, including proliferation, invasion, migration, and angiogenesis (Ramachandran and Hollenberg, 2008; Coughlin, 2001). PAR-1 increases cancer cell survival and invasion, which in turn stimulates tumor growth and metastasis (Nierodzik et al., 2006). Additionally, it is linked to the activation of downstream signaling pathways that support the aggressive behavior of gastric cancer cells, including PI3K-AKT and MAPK (Gurbuz and Karaca, 2013). It has been established that poor clinical outcomes, such as advanced tumor stages and lymph node metastasis, are associated with PAR-1 expression in gastric cancer tissues (Duvernay and Templeton, 2017). As a result, PAR-1 is considered a potential prognostic biomarker in gastric cancer. Targeting PAR-1 signaling pathways has emerged as a novel therapeutic approach, potentially limiting cancer progression and improving patient outcomes (Stein et al., 2003).

3.1 Structure and function of protease activator receptor 1

Protease activator receptor 1 (PAR-1), an associate of the G protein−coupled receptor (GPCR) family, theaters a vital role in cellular responses to protease-mediated signaling (Vu et al., 1991). Structurally, PAR-1 consists of a unique extracellular N-terminal domain, seven transmembrane helices, three intracellular loops, and an intracellular C-terminal tail (Xu et al., 1998). Serine proteases, such as thrombin, slice the N-terminal domain of PAR-1, exposing a new N-terminus that acts as a tethered ligand and activates the protein (Coughlin, 2000). The start of PAR-1 initiates intracellular signaling cascades through interaction with heterotrimeric G proteins, primarily Gαq (Kahn et al., 1999). This interaction sets off a series of cascading effects, such as the activation of phospholipase Cβ, which in turn generates diacylglycerol (DAG) and inositol trisphosphate (IP3), which causes intracellular calcium release and activates protein kinase C (PKC) (Brass and Shattil, 1985).

3.2 PAR-1 signaling pathways and their role in tumorigenesis

GPCR PAR-1 is critical for numerous physiological processes, including the growth of tumors (Coughlin, 2000). When serine proteases cleave PAR-1's N-terminal domain, a tethered ligand is revealed, which quandaries to the receptor and triggers it, and PAR-1 is activated (Vu et al., 1991). PAR-1 activation triggers various intracellular signaling pathways contributing to tumorigenesis (Macfarlane et al., 2001). One of the primary pathways involves the G protein−mediated start of phospholipase C (PLC), leading to the production of inositol trisphosphate (IP3) and diacylglycerol (DAG). IP3 induces calcium release from intracellular stores, while DAG triggers protein kinase C (PKC), which regulates cell proliferation, migration, and invasion (Hammes and Levin, 2011). Moreover, mitogen-activated protein kinase is activated upon PAR-1 activation (MAPK) pathways, including extracellular signal−regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 MAPK (Covic et al., 2002). These pathways are associated with cell survival, proliferation, and differentiation, which are all critical aspects of tumorigenesis. Apart from its immediate impact on cancerous cells, PAR-1 signaling also modifies the microenvironment surrounding tumors. Proangiogenic factors such as vascular endothelial growth factor (VEGF) are secreted by endothelial cells that have been activated by PAR-1, and this promotes angiogenesis (Even-Ram et al., 1998). This supports tumor growth and metastasis by ensuring adequate blood supply to the tumor. The role of PAR-1 in tumorigenesis extends to its involvement in the epithelial−mesenchymal transition (EMT), a process that enhances cancer cell invasion and metastasis (Acloque et al., 2009). PAR-1 activation contributes to EMT through various mechanisms, including regulating key transcription factors such as Snail, Slug, and Twist (Boire and Covic, 2018). The intricate signaling pathways activated by PAR-1 promote a tumorigenic microenvironment characterized by enhanced proliferation, migration, invasion, angiogenesis, and EMT (Nierodzik and Karpatkin, 2006). Understanding these pathways provides valuable insights into potential therapeutic strategies targeting PAR-1 in cancer treatment.

3.3 PAR-1 expression patterns in gastric cancer

In gastric cancer, the expression patterns of PAR-1 have been investigated to understand its role in disease progression and clinical significance. When compared with normal stomach mucosa, PAR-1 expression is frequently elevated in the tissues of gastric cancer (Zhang et al., 2012). This overexpression has been related to progressive tumor stages and poor overall survival of lymph node metastasis (Li et al., 2013). Additionally, studies have revealed correlations between PAR-1 expression and other clinicopathological factors, such as tumor differentiation and invasion depth (Wang et al., 2009). The activation of PAR-1 triggers intracellular signaling cascades that mediate cell proliferation, migration, and angiogenesis (Ramachandran and Hollenberg, 2008). The PAR-1 signaling axis involves pathways such as MAPK/ERK, PI3K/AKT, and NF-κB, which contribute to the aggressive behavior of gastric cancer cells (Fujimoto et al., 2005). Understanding the spatial and temporal expression patterns of PAR-1 in gastric cancer tissues provides valuable insights into its possible role as a prognostic lymph node metastasis and biomarker. Targeting PAR-1 and its associated signaling pathways could offer novel strategies for treating gastric cancer. PAR-1 has emerged as a potential prognostic indicator in gastric cancer because it promotes tumor growth, angiogenesis, and metastasis (Kataoka et al., 2003). Several studies have investigated the scientific relevance of PAR-1 expression in gastric cancer-affected role. Poor overall survival rates, lymph node metastasis, and advanced tumor stages have all been linked to high levels of PAR-1 expression (Shimizu et al., 2005; Nierodzik et al., 1998). The overexpression of PAR-1 has also been correlated with increased invasiveness and relocation of gastric cancer cells, contributing to the aggressive behavior of the disease (Abe et al., 2014).

4. Matrix metalloproteinase 1 and gastric cancer

MMP1, an associate of the matrix metalloproteinase family, plays an essential part in the progression and metastasis of gastric growth (Liotta et al., 1991). MMP1 is primarily responsible for collagen degradation, a significant ECM, thereby facilitating cancer cell attack and migration (Egeblad and Werb, 2002). In patients with gastric cancer, the overexpression of MMP1 has been linked to an advanced tumor stage, lymph node metastasis, and a lower overall existence rate (Gong et al., 2013). MMP1 expression in gastric cancer is controlled by various issues, including cytokines, growth factors, and hypoxia-inducible factors (Fig. 12.3) (Dufour et al., 2008a,b). This dysregulation contributes to the remodeling of the tumor microenvironment, favoring cancer cell dissemination (Li et al., 2007). Moreover, MMP1 has been concerned with angiogenesis by releasing proangiogenic factors from the ECM, thereby promoting the formation of new blood vessels to support tumor growth (Bergers and Coussens, 2000). Given its crucial role in gastric cancer progression, MMP1 has emerged as a potential therapeutic target. Efforts are being made to develop MMP1-specific inhibitors to halt its enzymatic activity and impede cancer cell invasion (Turk et al., 2000). However, the clinical translation of such inhibitors faces challenges in achieving specificity and avoiding off-target effects on normal tissue remodeling (Egeblad et al., 2010). Overexpression of MMP1 has been correlated with advanced tumor stage, lymph node metastasis, and poorer

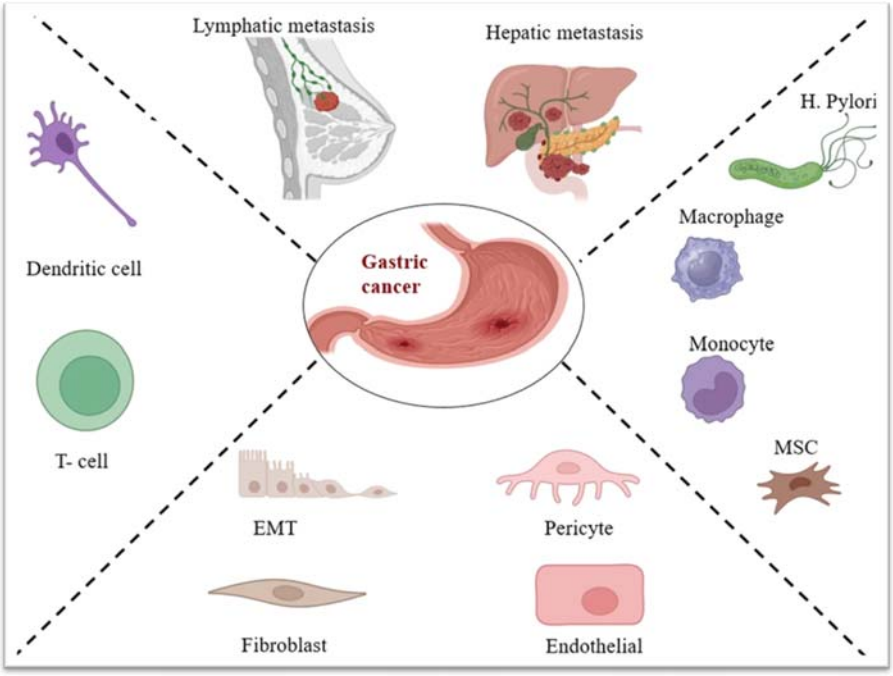

FIGURE 12.3 Matrix metalloprotease and gastric cancer.

survival in gastric cancer patients. MMP1 expression in gastric cancer is regulated by factors such as cytokines, growth factors, and hypoxia-inducible factors.

"Regulation of MMP1 expression in gastric cancer is influenced by cytokines, growth factors, and hypoxia-inducible factors." MMP1 is primarily responsible for collagen degradation, facilitating cancer cell invasion and migration.

4.1 Unraveling the role of matrix metalloproteinases in cancer invasion

MMPs are complicated in various stages of cancer progression, including angiogenesis, migration, and metastasis (Stetler-Stevenson and Yu, 2001). Their dysregulation can promote an environment conducive to tumor growth and spread (Egeblad and Werb, 2002). MMP1, also known as collagenase-1, plays a pivotal role in degrading collagen within the ECM (Zucker and Cao, 2014). Its substrate specificity is primarily for type I and III collagens (Iyer et al., 2012). Activation mechanisms involve proteolytic cleavage and posttranslational modifications (Van Wart and Birkedal-Hansen, 1990). Understanding MMP1's substrate preferences and activation pathways is crucial for deciphering its role in cancer invasion. In the gastric cancer microenvironment, MMP1 expression is upregulated by various factors, including growth factors, cytokines, and hypoxia (Kim et al., 2010). Its expression is frequently linked to advanced gastric cancer stages (Chu et al., 2016). Controlling elements such as transcription factors and epigenetic modifications contribute to MMP1 overexpression in this context (Hua et al., 2011). The overexpression of MMP1 in gastric cancer presents an opportunity

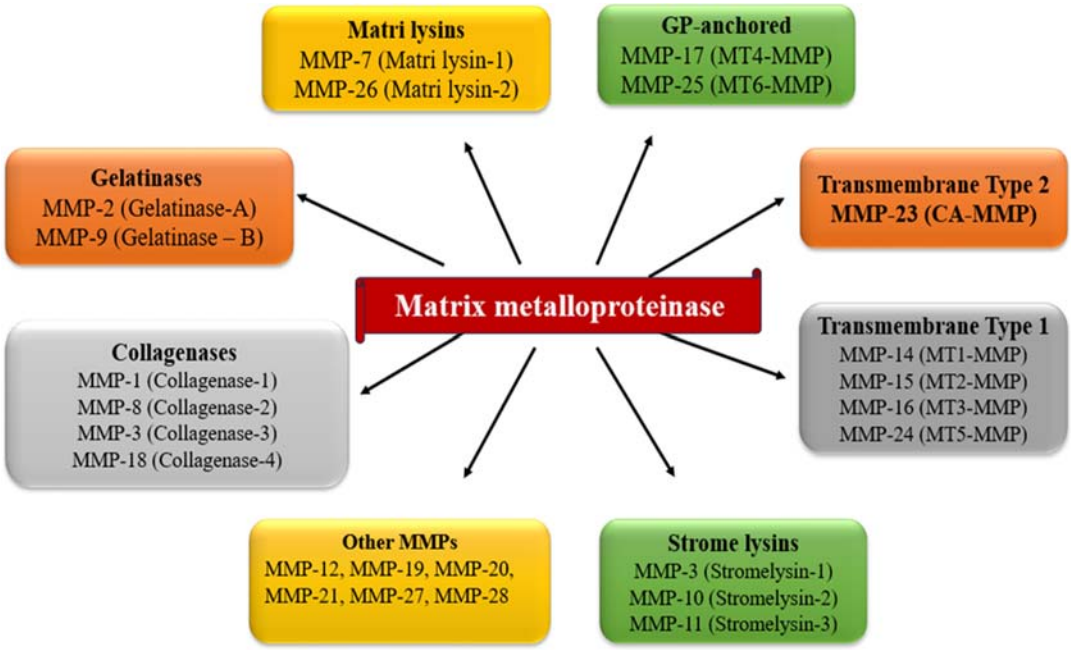

FIGURE 12.4 Role of matrix metalloprotease.

for targeted therapeutic strategies (Li et al., 2018). Inhibitors of MMPs have been explored in preclinical studies, aiming to attenuate cancer invasion and metastasis (Coussens et al., 2002). However, the challenge lies in achieving selective inhibition without affecting physiological processes (Fig. 12.4).

Matrix metalloproteinases (MMPs) are enzymes that play a crucial role in tissue remodeling by breaking down and modifying the extracellular matrix. They are involved in processes such as wound healing, tissue repair, and development. MMPs play a crucial role in cancer progression, including angiogenesis, migration, and metastasis. MMP1, also known as collagenase-1, specifically degrades type I and III collagens. It is upregulated in gastric cancer by growth factors, cytokines, and hypoxia. Overexpression of MMP1 in gastric cancer is associated with advanced stages of the disease. Targeting MMP1 presents a potential therapeutic strategy, although achieving selective inhibition remains challenging.

5. Prognostic and predictive significance of PAR-1 and MMP1 in gastric cancer

Elevated PAR-1 has been observed to be related to advanced tumor stage, lymph node metastasis, and deprived prognosis in gastric growth patients (Peng et al., 2008; Sun et al., 2015). Its presence in gastric cancer tissue correlates with shorter overall survival and disease-free survival rates (Lin et al., 2006). Overexpression of PAR-1 increases angiogenesis

and helps the tumor acquire invasive characteristics, which strengthens the tumor's aggressiveness (Xue et al., 2013a,b). MMP1, a member of the matrix metalloproteinase family, degrades the extracellular matrix and facilitates cancer cell invasion (McCawley and Matrisian, 2000). Elevated MMP1 expression has been linked to tumor invasion depth, lymph node metastasis, and progressive clinical stages in stomach cancer (Li et al., 2018). Its overexpression correlates with poor prognosis, contributing to increased local invasion and distant metastasis (Zheng et al., 2015). Moreover, MMP1 has been implicated in forming premetastatic niches, facilitating the establishment of secondary tumors (Zhang et al., 2019).

In addition to their prognostic value, PAR-1 and MMP1 also possess predictive significance in the context of gastric cancer therapy. PAR-1 expression has been associated with resistance to chemotherapy and radiotherapy in various cancer types (Prudova et al., 2010). Its upregulation can enhance cancer cell survival and resistance to apoptotic signals (Neumann et al., 2007). Thus, PAR-1 expression levels might serve as predictive markers for treatment response. Targeting PAR-1 may sensitize cancer cells to conventional therapies and improve treatment outcomes. MMP1's predictive significance lies in its potential as a therapeutic target for molecular interventions (Coussens et al., 2002). High MMP1 expression could indicate a more aggressive tumor phenotype that could benefit from MMP1-targeted therapies. Inhibiting MMP1 activity might impede tumor invasion and metastasis, potentially enhancing the effectiveness of existing treatment regimens.

6. Targeting PAR-1 and MMP1 for gastric cancer therapy

The action landscape for gastric cancer includes a combination of surgical resection, chemotherapy, targeted therapies, and immunotherapy (Smyth et al., 2016). Chemotherapy regimens often include fluoropyrimidines, platinum agents, and taxanes (Wagner et al., 2017). Targeted therapies such as trastuzumab for HER2-positive tumors and ramucirumab for VEGFR2-positive cases have improved outcomes (Bang et al., 2010; Wilke et al., 2014). Immunotherapy agents such as checkpoint inhibitors are being explored for subsets of gastric cancer patients (Fuchs et al., 2018). PAR-1 signaling promotes cancer cell survival, attack, and angiogenesis (Darmoul et al., 2004). Preclinical studies have established promise for constraining tumor growth and metastasis by targeting PAR-1 with small molecule inhibitors or monoclonal antibodies (Shi et al., 2004). Strategies include blocking PAR-1 activation and downstream signaling pathways (Ramachandran and Hollenberg, 2008). MMP1 is a critical enzyme in cancer invasion and metastasis by degrading ECM components (Egeblad and Werb, 2002). Developing MMP1 inhibitors has been challenging due to the difficulty of achieving selectivity and avoiding unintended off-target effects on other MMP family members (Coussens et al., 2002). However, recent advancements in rational drug design and high-throughput screening provide new avenues for MMP1-targeted therapies (Ochiya et al., 2018). Simultaneous targeting of PAR-1 and MMP1 presents a promising approach to enhance therapeutic efficacy (Dufour et al., 2008a,b). Combining PAR-1 and MMP1 inhibitors may synergistically disrupt tumor growth, invasion, and metastasis (Noel et al., 2008). Cotargeting strategies require careful consideration of dosing and timing to maximize benefits while minimizing adverse effects (Whittaker et al., 1999).

7. Future directions and challenges

As protease research in gastric cancer continues to evolve, several unexplored aspects, research avenues, and challenges warrant attention for deeper understanding and efficacious clinical application. While the functions of MMP1 and PAR-1 in gastric cancer are clarified, numerous unexplored facets remain. Future investigations could delve into the intricate cross-talk between these proteases and other signaling pathways, shedding light on potential synergies or antagonisms. Exploring their involvement in different subtypes of gastric cancer and their interaction with the tumor microenvironment could provide valuable insights into disease progression (Kessenbrock et al., 2010; Camerer et al., 2010). Advancing from the discovery phase, preclinical and clinical studies hold the key to translating the potential of targeting PAR-1 and MMP1 into effective therapies. In-depth mechanistic studies using advanced model systems can unravel the precise molecular mechanisms underlying their contributions. Rigorous clinical trials assessing the safety, efficacy, and long-term outcomes of therapies targeting these proteases are essential to realizing their clinical utility (Bang et al., 2010; Turk et al., 2000). Translating research findings into clinical practice poses challenges such as developing clinically relevant biomarkers, optimizing treatment regimens, and ensuring patient safety. Addressing potential off-target effects and resistance mechanisms is crucial for the successful clinical implementation of therapies targeting PAR-1 and MMP1. Collaborative efforts among researchers, clinicians, and regulatory agencies are essential to navigate these hurdles (Le et al., 2017; Ajani et al., 2016).

8. Conclusion and future perspectives

Proteases are pivotal in cancer progression, impacting various stages of tumor development, invasion, and metastasis. In gastric cancer, PAR-1 and MMP1 are significant contributors, driving aggressive behavior and poor clinical outcomes. Their roles as prognostic indicators and potential therapeutic targets offer new avenues for personalized treatment strategies in gastric cancer. Advances in understanding the molecular mechanisms of PAR-1 and MMP1 signaling provide opportunities to develop targeted therapies to limit tumor growth and metastasis. As we navigate the complexities of protease-driven tumorigenesis, continued research efforts are crucial to uncover the nuances of their roles, interactions, and clinical applications. By elucidating the intricate web of protease-related pathways and their impact on the tumor microenvironment, we pave the way for novel therapeutic interventions that potentially transform the landscape of gastric cancer treatment. Collaborative endeavors between researchers, clinicians, and pharmaceutical developers are essential to bridge the gap between the bench and bedside, ultimately translating promising discoveries into better patient consequences.

In the future, further exploration of PAR-1 and MMP1 in the context of gastric cancer holds immense promise and a set of key challenges. Research should focus on unraveling the intricate cross-talk between these proteases and their interactions with other signaling pathways and the tumor microenvironment, shedding light on potential synergistic or antagonistic effects. In-depth mechanistic studies, using advanced model systems, are essential to uncover the precise molecular mechanisms underlying their contributions to

cancer progression. Rigorous clinical trials are necessary to assess the safety, efficacy, and long-term outcomes of therapies targeting these proteases. To successfully translate research findings into clinical practice, efforts must be directed at developing clinically relevant biomarkers, optimizing treatment regimens, and addressing potential off-target effects and resistance mechanisms. Collaborative endeavors among researchers, clinicians, and regulatory agencies are vital to navigate these challenges and ultimately transform the landscape of gastric cancer treatment with targeted therapies that offer personalized and more effective options for patients.

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CHAPTER

13

Matrix metalloproteases as diagnostic and prognostic biomarkers in cancer

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1. Introduction

Matrix metalloproteinases (MMPs) are a member of the metazincin protease superfamily. Metazinicins have been classified into six families because of their dependence on zinc ions for their enzymatic activity. These are ADAMs (a disintegrin and metalloproteinase or adamalysin), ADAMTSs (ADAMs having thrombospondin motif), astacins, MMPs, pappalysins, and serralysins (Tokito and Jougasaki, 2016; Wang et al., 2021). MMPs were initially discovered in 1949 as tumor-promoting depolymerizing enzymes. They induced tumor formation by increasing the fluidity of connective tissue stroma, which includes tiny blood vessels. In 1962, Gross and Lapiere (1962) identified and analyzed MMP collagenase as the enzyme in charge of tadpole tail resorption. Over the years, various MMP enzymes have been identified and purified, leading to a deeper understanding of their functions in different biological contexts. Notably, MMPs are found not only in animals but also in archaebacteria, bacteria, viruses, nematodes, and plants, highlighting their evolutionary significance and widespread presence in living organisms (Laronha and Caldeira, 2020).

1.1 Structure of MMP

The molecular structures of most MMPs are similar consisting of *N-terminal signal peptide:* MMPs typically have an N-terminal signal peptide that guides them to secretory pathways

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within the cell. In the case of membrane-type MMPs (MT-MMPs), this signal peptide directs them to the cell membrane where they are anchored. *Propeptide:* MMPs are produced as inactive zymogens or pro-MMPs. The propeptide contains a conserved amino acid sequence, PRCGXPD, which wraps around a cysteine residue. This cysteine residue has a thiol (SH-) group that can bind to a zinc ion (Zn^{2+}) in the catalytic region of the enzyme. This interaction keeps the enzyme in an inactive or latent form. *Catalytic motif:* The catalytic motif of MMPs contains the conserved HEXXHXXGXXH sequence. Within this motif, the three histidine residues (H) are involved in binding to a zinc ion (Zn^{2+}). This zinc ion, in turn, interacts with the thiol group of the cysteine residue in the propeptide, stabilizing the inactive form of the enzyme (Fig. 13.1A).

Structure and classification of MMPs are shown in Fig. 13.1.

Activation of MMPs often involves the removal of the propeptide, allowing the enzyme to adopt an active conformation and participate in various biological processes such as tissue remodeling and degradation of extracellular matrix (ECM) components. A proline-rich hinge/linker domain and a hemopexin-like region located at the C-terminus involved in modulation of enzymatic activity, substrate specificity, and MMP localization (Galea et al., 2014; Gonzalez-Avila et al., 2022; Nguyen et al., 2013).

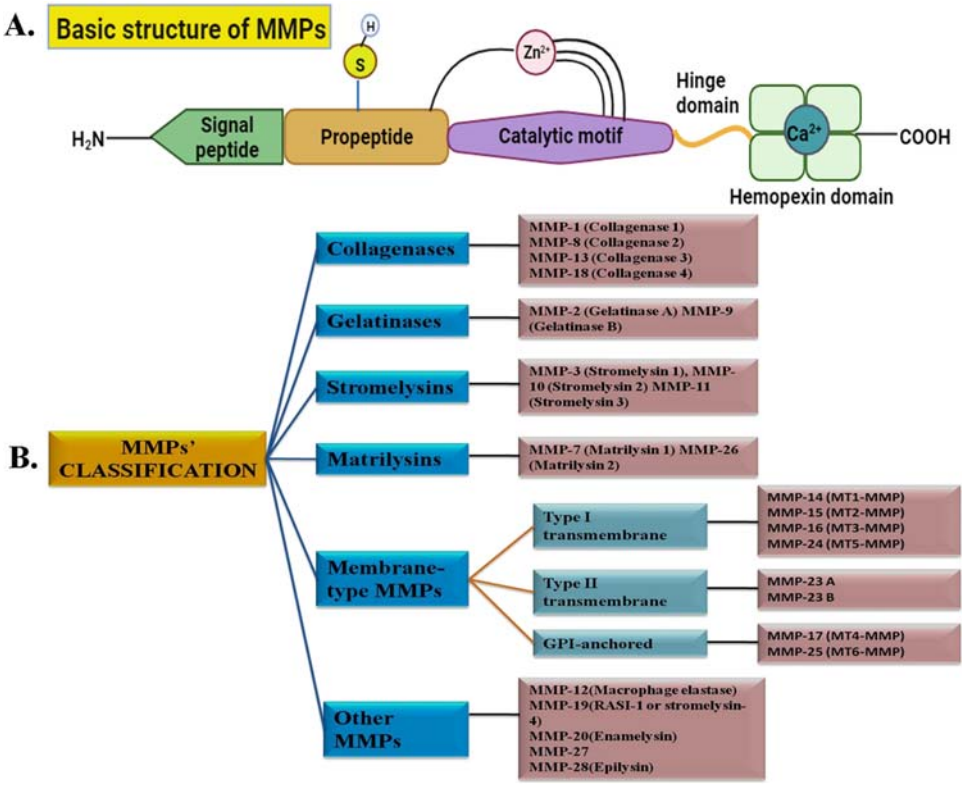

FIGURE 13.1 Matrix metalloproteinases (MMPs): (A) Basic structure of MMPs. (B) Classifications of MMPs.

1.2 Types of MMPs and their functions

It is now known that vertebrates have 28 MMPs; however, only 24 of them have been identified in humans (Gonzalez-Avila et al., 2022). Based on bioinformatic analysis, MMPs can be classified into five different types (Cerofolini et al., 2019). (**1**) MMPs not regulated by furin: MMP-1, -3, -7, -8, -10, -12, -13, -20, and -27. (**2**) MMPs with three fibronectin-like inserts: MMP-2 and MMP-9. (**3**) MMPs anchored to the cellular membrane by GPI moiety: MMP-11, -17, and -25. (**4**) MMPs with a transmembrane domain: MMP-14, -15, -16, and -24. (**5**) Other MMPs: MMP-19, -21, -23, -26, and -28. Similarly, based on domain organization and substrate they target, MMPs are categorized as collagenases (target collagen), gelatinases (degrade gelatin, a denatured form of collagen), stromelysins (degrade a variety of ECM components), matrilysins (degrade components of the ECM), membrane-type MMPs (anchored to the cell membrane and have diverse substrate specificities), and other MMPs (domain organizations and have diverse substrate specificities) depicted in Fig. 13.1B.

Collagenases are enzymes specialized in breaking down various components of the ECM, especially fibrillar collagen types I, II, III, IV, and XI into two distinct fragments, 1/4 C-terminal and 3/4 N-terminal. The cleavage of fibrillar collagen occurs in two stages: First, collagenases unwind the tightly bound triple helical structure of collagen, making it accessible for enzymatic action followed by hydrolyzing specific peptide bonds within the collagen molecules, breaking them down into distinctive fragments (Cui et al., 2017; Fischer et al., 2019; Nagase et al., 2006).

Gelatinases play vital roles in physiological processes such as ECM degradation, osteogenesis, and wound healing. They can degrade various substrates, including gelatin, collagen types IV, V, VIII, X, XI, XIV, elastin, proteoglycan core proteins, fibronectin, laminin, fibrillin-1, and precursor cytokines such as TNF-α and IL-1β. MMP-2 and MMP-9 contain fibronectin type II domains, enabling them to bind and degrade gelatin, collagen, and laminin. MMP-2, primarily a gelatinase, also exhibits collagenase-like activity. MMP-9 acts as both collagenase and gelatinase, crucial for processes such as embryonic development, angiogenesis, inflammation, and tumor metastasis. Elevated gelatinase expression is linked to various cancers, indicating their significance in cancer progression and metastasis (Klein and Bischoff, 2011; MURPHY and NAGASE, 2008; Mannello and Medda, 2012).

Stromelysins share a domain structure similar to collagenases but do not cleave interstitial collagen. Despite this, stromelysins have distinct substrate preferences, setting them apart from collagenases and highlighting their unique role in ECM remodeling. MMP-3 and MMP-10, closely related both in structure and substrate specificity, exhibit functional similarities. In contrast, MMP-11 differs significantly from MMP-3 and MMP-10. It features a distinctive 10-amino acid insert (RXRXKR) between the pro- and catalytic domains, crucial for intracellular activation. This insert is recognized by the Golgi-associated proteinase furin, leading to MMP-11 activation within the cell (Cox, 2021; Cui et al., 2017; Nagase et al., 2006).

Matrilysins are distinct MMPs due to the absence of a hemopexin domain, a characteristic feature found in many other MMPs, crucial for substrate recognition and binding. Matrilysins exhibit specificity in amino acid sequence, particularly concerning threonine residues near the Zn^{2+}-binding site. This unique sequence specificity likely affects their interactions with substrates and enzymatic activity (MURPHY and NAGASE, 2008; Mannello and Medda, 2012).

MT-MMPs (membrane-type matrix metalloproteinases) possess a furin-like proprotein convertase recognition site (RX[R/K]R) in their C-terminal prodomain, enabling their activation through proteolytic removal of this domain. Intracellular activation of MT-MMPs leads to the expression of active enzymes on the cell surface (Cui et al., 2017). They can be categorized into three groups: type I transmembrane MT-MMPs, type II transmembrane MT-MMPs, and GPI-anchored proteins (MURPHY and NAGASE, 2008; Mannello and Medda, 2012). Type I transmembrane MT-MMPs have a long cytoplasmic tail of about 20 amino acids following the transmembrane domain, playing a role in intracellular signaling and interactions. MT-MMPs also feature an eight-amino-acid sequence inserted into the catalytic domain, influencing the active site cleft's structure, potentially affecting substrate specificity and enzymatic activity. Other MT-MMPs include MMP-12, MMP-19, MMP-20, MMP-27, and MMP-28 (Cui et al., 2017).

1.3 Physiological functions of MMPs

The ECM is a vital, noncellular structure present in all body tissues, providing crucial support to cells during their development (Hynes, 2009). MMPs play a significant role in ECM degradation and remodeling. They are involved in processing growth factors and their receptors within the ECM, which is essential for wound healing. MMPs regulate angiogenic factors, either stimulating or preventing angiogenesis (Schultz and Wysocki, 2009). They release growth factors such as TGFβ and VEGF from the ECM. TGFβ is a multifunctional cytokine, while VEGF regulates angiogenesis by promoting blood vessel growth and permeability (Bergers et al., 2000; Mott and Werb, 2004; Park et al., 1993). They also play essential roles in various cellular processes, including angiogenesis, aging, blastocyst implantation, embryogenesis, wound healing, tissue repair, bone remodeling, cell proliferation, migration, apoptosis, differentiation, and reproductive events such as ovulation and endometrial proliferation (Alaseem et al., 2019; Ricard-Blum and Salza, 2014). While some MMPs function intracellularly, the mechanisms behind their initial release and reentry into cells remain unclear. Certain MMPs can enter cells through endocytosis, utilizing clathrin-dependent or clathrin-independent pathways (Cauwe and Opdenakker, 2010). In clathrin-dependent mechanisms, cargo proteins bind to adaptor proteins, forming clathrin-coated vesicles that are taken up by cells (Grant and Donaldson, 2009; Traub, 2009). Clathrin-independent mechanisms involve macropinocytosis, phagocytosis, caveolae, or flotillin-dependent processes (Grant and Donaldson, 2009; Swanson, 2008). For instance, MMP7 enters neurons through a clathrin-dependent mechanism, associated with the processing of a 25-kDa synaptosomal-associated protein (Szklarczyk et al., 2007). Low-density lipoprotein-related protein 1 (LRP1) allows MMPs such as MMP2, MMP9, and MMP13 to enter cells depicted in Fig. 13.2 (Raggatt et al., 2006; Van Den Steen et al., 2006).

Role of MMPs in tumor progression is shown in Fig. 13.2.

Reactive oxygen species (ROS) are crucial for immunological responses and maintaining metabolism, generated by various chemical oxidants such as superoxide anion (O2•-), hydroxyl radicals (•OH), and hydrogen peroxide (H_2O_2) (Muri and Kopf, 2021; Sies and Jones, 2020). Nitric oxide (NO•) is a vital regulatory molecule, but an imbalance between O2•- and NO• can lead to peroxynitrite (ONOO-) formation, causing nitrosative stress and damaging lipids, proteins, and DNA (Brandes et al., 2009; Pacher et al., 2007). ROS cause

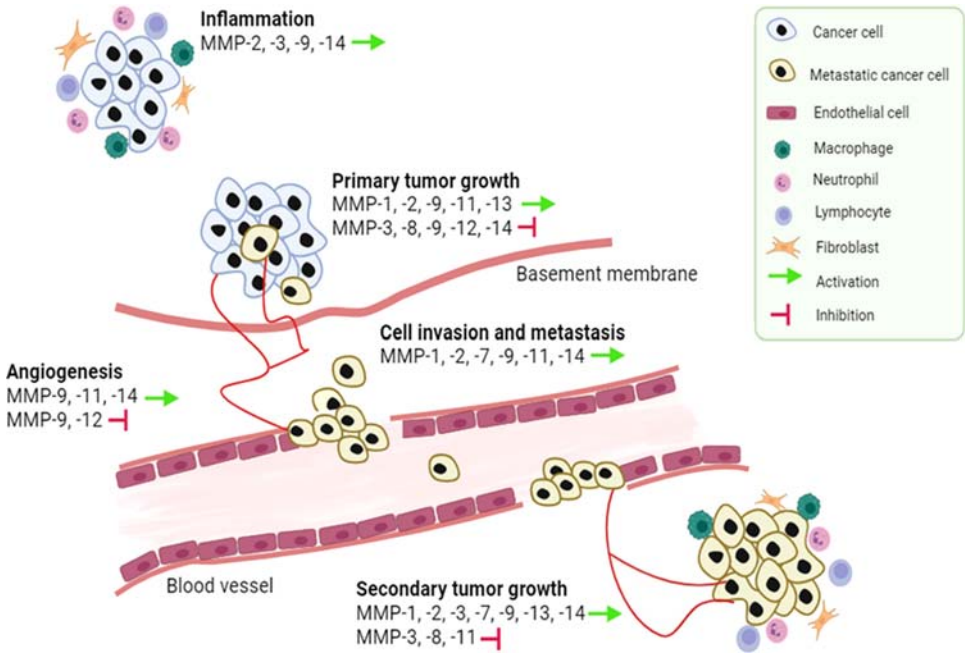

FIGURE 13.2 MMPs in tumor progression. Function of MMPs in tumorigenesis, MMPs having both functions activation as well as inhibition of tumorigenesis depending on the cancer progression stage.

posttranslational modifications, affecting MMPs by oxidizing their thiol groups (Ali and Schulz, 2009; Martínez and Andriantsitohaina, 2009; Okamoto et al., 2001). Peroxynitrite activates MMPs by S-glutathiolation or S-nitrosylation of specific cysteines, forming disulfide S-oxide bonds (Jacob-Ferreira et al., 2013; Viappiani et al., 2009). This activation occurs during stress or immunological responses. For instance, hypochlorous acid activates MMPs during neutrophil activation via myeloperoxidase (Fu et al., 2001; Meli et al., 2003). Dysregulated MMP activity can lead to various pathological conditions, emphasizing the importance of MMP regulation (Amălinei et al., 2010; Tallant et al., 2010).

2. Natural inhibitors of MMPs

Tissue inhibitors of metalloproteinases (TIMPs) are crucial in regulating MMP activity, preventing excessive tissue damage (Lambert et al., 2004). TIMPs include four members: TIMP-1 (a 28-kDa soluble glycosylated protein), TIMP-2 (a 21-kDa soluble nonglycosylated protein), TIMP-3 (a 24–27-kDa glycosylated protein attached to the cell surface and ECM), and TIMP-4 (a 22-kDa glycosylated protein), expressed in various body organs to inhibit overexpressed MMPs (Cabral-Pacheco et al., 2020). These TIMPs have molecular masses ranging from 22 to 28 kDa (Khokha et al., 2013) and exhibit similar fundamental structures, resembling wedges composed of two major domains: an N-terminal domain of approximately 125 amino acids and a C-terminal domain of about 65 amino acids (Brew et al.,

2000; Masciantonio et al., 2017). Six conserved disulfide bonds bind these domains together, with each domain mediating distinct functions (Brew and Nagase, 2010). TIMPs regulate metalloproteinase activity by interacting with their catalytic sites. The N-terminal domain of TIMPs blocks the Zn^{2+} molecule in the active site, inhibiting metalloproteinase activity at a 1:1 ratio (Jayawardena et al., 2019; Masciantonio et al., 2017). Although initially thought to have a partial role, the C-terminal domain of TIMPs also participates in protein−protein interactions. TIMP1 and TIMP3 can interact with proMMP9, while TIMP2, TIMP3, and TIMP4 interact with proMMP2. TIMPs, except TIMP-2, are inducible proteins, performing various functions, including MMP activation. TIMP-2 combines with MMP-14 and pro-MMP-2, forming an activation complex. Two MMP-14 molecules initially dimerize on the cell surface, and TIMP-2 binds to MMP-14 and pro-MMP-2. MMP-14 disrupts the propeptide of MMP-2, partially activating it (Itoh, 2015). The full enzymatic activity of MMP-2 is achieved through its autoproteolysis. TIMP-1 binds to the hemopexin motif of pro-MMP-9 and interacts with the catalytic site of MMP-3, forming a tertiary complex. Active MMP-3 molecules release pro-MMP-9 from this complex, followed by propeptide cleavage by free MMP-3. MMP-1 can also release pro-MMP-9 by forming a complex with pro-MMP-9, TIMP-1, and MMP-1 (Ogata et al., 1995).

3. Involvements of MMPs in cancer progression

Tumor cells develop through various processes such as genome instability, immortality, uncontrolled cell division, immune system evasion, induction of angiogenesis, resistance to cell death, altered metabolism, promotion of inflammation, and mechanisms leading to metastasis (Hanahan and Weinberg, 2011). The main tumor comprises diverse cells with distinct genetic and phenotypic traits (Gerdes et al., 2014). Some cells acquire unique characteristics, enabling them to break away from the main tumor and initiate metastasis. These traits include resistance to anoikis (cell death when detached), metabolic adaptation to the tumor microenvironment, and cell plasticity through epithelial−mesenchymal transition (EMT), and allowing invasion into surrounding tissues (Celià-Terrassa and Kang, 2016). Metastatic cells detach, enter blood or lymphatic vessels, evade the immune system, and attach to endothelial cells in distant organs. They may revert to their original epithelial state (mesenchymal−epithelial transition) and form micrometastases that can grow under suitable conditions.

Tumors are complex, heterogenous, and interact with their microenvironment (Maley et al., 2017; Quail and Joyce, 2013). Dysregulation of cancer-related signaling affects various processes such as immunological control, blood vessel development, metabolism, and growth (Hiam-Galvez et al., 2021). MMPs play a significant role in these dysregulated mechanisms (Egeblad and Werb, 2002; Piperigkou et al., 2021). MMPs have been extensively studied in cancer due to their ability to modify the basement membrane and trigger cell invasion, leading to increased metastasis (Piperigkou et al., 2021; Young et al., 2019). Thus, MMP overexpression is strongly correlated with cancer development and clinical prognosis.

3.1 Matrix remodeling and cell invasion

The ECM serves as the fundamental structural framework of all tissues and organs, maintaining tissue integrity when the production of ECM is balanced with its destruction, or the equilibrium of ECM turnover. However, cancer disrupts ECM homeostasis at biochemical, biological, and structural levels due to its dynamic nature and dysregulated development (Cox, 2021). Importantly, ECM may be pro- or antitumorigenic and is profoundly dysregulated in malignancies. For instance, elevated expression and activity of MMP8 is associated with positive outcomes in skin and squamous cell carcinoma (Juurikka et al., 2021; Korpi et al., 2008), whereas it has negative outcomes in gastric, hepatic, and ovarian cancers. A number of MMPs have the ability to cleave ECM proteins and release growth factors, cytokines, and their cell surface-bound receptors in a targeted manner. This can affect tissue turnover and overall ECM integrity. There is compelling evidence that either MT1-MMP, MT2-MMP, or MT3-MMP alone is sufficient to drive ECM transmigration and invasion and may be the major MMPs driving ECM remodeling, despite the fact that many MMPs have been shown to remodel the ECM (Piperigkou et al., 2021; Shimoda et al., 2021).

3.2 Angiogenesis

Angiogenesis, or the creation of new blood vessels from preexisting vascular networks, is a crucial process in the development of cancer because tumors have high blood sugar and nutritional demands (De Palma, Biziato and Petrova, 2017). Low oxygen availability, or hypoxia, is a crucial component of tumor angiogenesis. To increase vascular formation and obtain the proper oxygen supply, hypoxic cancer cells secrete more growth factors, such as VEGFA (Potente et al., 2011). Moreover, MMPs have been directly linked to controlling angiogenesis in the majority of cancers. In premalignant lesions, broad-spectrum inhibition with batimastat prevented the angiogenic switch (Bergers et al., 1999). Activated endothelial and immune cells produce MMPs and other proteases that remodel the ECM during sprouting angiogenesis. MMPs frequently affect the vascular BM in tumors, which can be irregular, discontinuous, and loosely connected to pericytes and endothelial cells. This leads to an increase in vascular leakiness, which in turn increases the intravasation and metastasis of cancer cells into the vascular system (De Palma, Biziato and Petrova, 2017; Piperigkou et al., 2021).

3.3 Apoptosis

Cancer cells need to avoid programmed cell death to survive. Extracellular receptors, such as the FAS receptors, have the ability to trigger apoptosis. This can lead to the degradation of nuclear DNA and other substrates as well as an intracellular caspase-mediated proteolytic cascade (Kessenbrock et al., 2010; Young et al., 2019). Research has demonstrated that MMPs impede the process of inducing apoptosis in cancerous cells. For instance, MMP-7 was demonstrated to cleave Fas ligand and showed protective effect by lowering cell death in a doxorubicin-mediated cell death using cancer cell lines SK-N-MC and SW-480 (Mitsiades et al., 2001).

4. MMPs as prognostic and diagnostic biomarkers in cancer

MMPs play a significant role in various types of cancer, influencing the aggressiveness and prognosis of the disease. Specific MMPs are associated with increased expression levels, indicating a more aggressive form of the disease. Diagnostic markers in cancer and MMPs play distinct but interconnected roles in cancer progression. Diagnostic markers are used for the early detection, diagnosis, and prognosis of cancer, while MMPs are enzymes involved in the degradation of the ECM, a crucial process in tumor invasion and metastasis. These findings underscore the potential utility of MMPs as diagnostic and prognostic markers in cancer, and they provide valuable insights into the mechanisms underlying cancer progression (Table 13.1).

4.1 Breast cancer

Breast cancer stands as the most prevalent malignancy and the leading cause of cancer-related fatalities among women globally. It is categorized into four primary subtypes based on the status of hormone receptors (estrogen receptor [ER] or progesterone receptor [PR]) and human epidermal growth factor receptor 2 (HER2): hormone receptor-positive/HER2-negative (often referred to as luminal A), hormone receptor-positive/HER2-positive (luminal B), hormone receptor-negative/HER2-positive (HER2-positive or HER2-enriched), and triple-negative breast cancer (TNBC) (Kwon, 2023). MMPs have the potential to function as biomarkers for various aspects of breast cancer management, such as diagnosis, prognosis, early detection, risk evaluation, and assessment of therapeutic effectiveness. Elevated concentrations of MMP-1, MMP-9, and MMP-13 have been observed in the blood, urine, and breast tumor tissues of individuals with breast cancer (Roy et al., 2020). MMP-1 levels were significantly higher in the tumor and tumor stromal cells of lymph node metastatic breast cancer tissues than in those of nonmetastatic tissues, MMP-2 and MMP-9 have been linked to the invasive potential of breast tumors (Wang et al., 2019). High levels of these MMPs are correlated with a more aggressive disease course and poorer prognosis. These findings highlight the clinical significance of MMPs in breast cancer as indicators of disease aggressiveness and prognostic factors. BRCA1, when highly expressed, is associated with a worse prognosis in untreated patients and is detected using immunohistochemistry (James et al., 2007).

4.2 Colorectal cancer

MMP-2, MMP-3, MMP-7, and MMP-9 are involved in disease progression, invasion, and metastasis. MMP-2 and MMP-3 are implicated in colorectal cancer progression and are associated with the invasive behavior of colorectal cancer cells. Increased expression of MMP-2 is linked to a more aggressive form of colorectal cancer.MMP-7 has emerged as a prognostic marker, and elevated expression of MMP-7 is associated with advanced disease and is linked to a poorer prognosis (Said et al., 2014). The clinical significance of MMP-7 underscores its role in predicting disease outcomes and guiding therapeutic decisions. MMP-9 is also

TABLE 13.1 Different MMPs employed as prognostic and diagnostic biomarkers in various type of cancer.

Type of cancer	MMPs involved	Marker expression	References
Breast cancer	MMP-1, -2, -3, -7, -8, -9, -11, -13, -14, -15, -16, -24, -25	These MMPs are highly expressed in triple-negative breast cancer tissues compared to estrogen receptor-positive (ER+) and human epidermal growth factor receptor 2-positive (HER2+) breast cancer tissues.	James et al. (2007), Roy et al. (2020), Wang et al. (2019)
Lung cancer	MMP-1, -2, -7, -9, -10, -11, -13, -14, -15, -16, -24, -28	Higher expression of these MMPs is associated with a worse prognosis, including reduced overall survival.	Wei (2023)
Colorectal cancer	MMP-2, -7, -9, -13, -14, -15, -20, -23	Increased expression of MMP-2 is linked to a more aggressive form of colorectal cancer. Elevated expression of MMP-9 is linked to a more aggressive disease.	Said et al. (2014)
Ovarian cancer	MMP-1, -2, -8, -9, -13, -14, -15, -16, -17, -19, -20, -24, -26	Expression levels of these MMPs have been correlated with disease progression and prognosis. Specifically, high expression levels of MMPs-19 and MMP-20 are associated with a poor prognosis in ovarian cancer.	Al-Alem and Curry (2015), Carey et al. (2021), Kenny and Lengyel (2009), Wang et al. (2019)
Brain cancer	MMP-1, -2, -3, -7, -9, -10, -11, -13, -14	Overexpressed in glioblastoma tumors, and their increased expression levels are associated with more aggressive disease and poorer patient outcomes.	Hagemann (2012), Roy et al. (2020)
Hepatocellular cancer	MMP-2, -9, -1, -3, -7, -13, -14, -15, -16, -17, -24, -25	Increased expression of MMP-2 is associated with the invasive behavior of HCC cells and the breakdown of the extracellular matrix, which allows cancer cells to infiltrate and metastasize. Upregulation of MMP-9 is linked to the aggressive behavior of the disease. The elevated MMP-7 and MMP-8 expression in HCC associated tumor cell invasion and migration, which leads to poor prognosis.	Scheau et al. (2019)
Pancreatic cancer	MMP-1, -2, -3, -9, -7, -10, -11, -13, -14, -15	Elevated MMP-2 expression linked to a more aggressive form of pancreatic cancer. Elevated expression of MMP-7 is linked to a more aggressive disease. Upregulated expression of MMP-14 is connected to the ability of PDAC to invade and metastasize.	Slapak et al. (2020)

(*Continued*)

TABLE 13.1 Different MMPs employed as prognostic and diagnostic biomarkers in various type of cancer.—cont'd

Type of cancer	MMPs involved	Marker expression	References
Renal cell carcinoma	MMP-2, -7, -9, -10, -14, -15, -16, -24	Increased expression of MMP-2 and MMP-9 is linked to a more aggressive form of renal cell carcinoma. Ac as a prognostic marker.	Kudelski et al. (2023)
Bladder cancer	MMP-1, -2, -3, -9, -7, -10, -11, -12, -13, -14	Increased expression of MMP-2 and MMP 9 is linked to a more aggressive form of bladder cancer.	Kudelski et al. (2023); Nutt et al. (2003)
Gastric cancer	MMP-2, -7, -9, -11, -13, -14, -15, -16, -24	Increased expression of MMP-2 is linked to a more aggressive form of gastric cancer. Elevated expression of MMP-9 is linked to a more aggressive disease.	Verma (2014)
Prostate cancer	MMP-2, -7, -9, -14, -15, -16, -26	In prostate cancer, MMPs have been studied for their involvement in tumor progression, invasion, metastasis, and angiogenesis.	Gong et al. (2014); Xie et al. (2016)
Melanoma cancer	MMP-1, -2, -3, -7, -9, -10, -13, -14, -26	Increased expression of MMP-1 is linked to a more aggressive form of melanoma. Elevated expression of MMP-2 is linked to a more aggressive form of melanoma. Increased expression of MMP-9 is linked to a more aggressive form of melanoma.	Napoli et al. (2020)
Osteosarcoma	MMP-1, -2, -3, -9, -7, -11, -12, -13, -14	Increased expression of MMP-2 is associated poor prognosis. Elevated expression of MMP-9 is linked to a more aggressive disease. Upregulated expression of MMP-14 is connected to the ability of osteosarcoma to spread to other sites in the body.	Bjørnland et al. (2005), Thanapprapasr et al. (2014)

associated with colorectal cancer progression and is involved in the invasive properties of colorectal cancer cells. Elevated expression of MMP-9 is linked to a more aggressive disease.

4.3 Lung cancer

Particularly, in non−small-cell lung cancer (NSCLC), MMPs have been investigated as prognostic markers. MMP-2 and MMP-9 are two important MMPs associated with lung cancer invasion and metastasis. These MMPs are known to degrade ECM components, promoting the invasion of lung cancer cells (Merchant et al., 2017). MMP-2 and MMP-9 are known to play a role in cancer cell invasion and metastasis. MMP-7 (matrilysin) has been implicated in lung tumorigenesis. It is associated with the breakdown of basement membranes and the

promotion of tumor growth in the lung (Wei, 2023). MMP-12 (macrophage metalloelastase) has a dual role in lung cancer. It can be both protective and detrimental. MMP-12 is associated with the degradation of elastin and ECM, which can facilitate tumor growth. However, in some cases, MMP-12 overexpression has been linked to increased survival, possibly due to its role in inhibiting angiogenesis (Qu et al., 2009). Higher expression of these MMPs is associated with a worse prognosis, including reduced overall survival. The clinical significance of MMPs in lung cancer highlights their potential as indicators of disease progression and as factors influencing patient outcomes.

4.4 Gastrointestinal cancers

It includes gastric and esophageal cancers. MMPs have been linked to tumor invasion and metastasis. MMP-9, in particular, has shown clinical significance as a prognostic marker in these cancers. Understanding the levels of MMPs in gastrointestinal tumors can help predict the risk of aggressive disease behavior and influence clinical decisions (Verma, 2014).

4.5 Ovarian cancer

MMPs are linked to the occurrence, development, invasion, and metastasis of ovarian cancer. Ovarian cancer is a type of cancer that starts in the ovaries and can be aggressive, often diagnosed at an advanced stage. MMPs have clinical relevance such as MMP-2, MMP-7, MMP-9, and MMP-14. MMP-19 and MMP-20 are associated with tumor invasion and metastasis. These are specific MMPs that have been identified as potential biomarkers of ovarian cancer, but in MMP-9, expression in stromal cells appears to have clinical significance, serving as a potential biomarker for disease progression and prognosis in ovarian cancer patients (Carey et al., 2021). In ovarian cancer, increased expression of MMP-9 in stromal cells (connective tissue cells in the tumor microenvironment) has been noted. This is in contrast to tumor cells, where MMP-9 expression may not be as strongly correlated with disease progression (Roy et al., 2020). Their expression levels have been correlated with disease progression and prognosis. The clinical significance of MMPs in ovarian cancer underscores their potential as markers for predicting patient outcomes and the need for tailored treatment approaches (Al-Alem and Curry, 2015).

4.6 Pancreatic ductal adenocarcinoma

Pancreatic cancer is a highly aggressive and often deadly form of cancer that originates in the tissues of the pancreas. The ECM plays a significant role in the development and progression of pancreatic cancer, and MMPs are a family of enzymes that are involved in ECM remodeling. MMP-2, MMP-7, and MMP-14 are shown to potentiate tumor growth and/or metastasis. MMP-2 is implicated in pancreatic ductal adenocarcinoma (PDAC) progression and is associated with the ability of cancer cells to invade surrounding tissues. Increased expression of MMP-2 is linked to a more aggressive form of pancreatic cancer. MMP-7 is also associated with PDAC progression and is involved in the invasive properties of pancreatic cancer cells. Elevated expression of MMP-7 is linked to a more aggressive disease. MMP-14 has been implicated in the progression of PDAC. It plays a role in the

degradation of the ECM and is associated with the invasive behavior of cancer cells. Upregulated expression of MMP-14 is connected to the ability of PDAC to invade and metastasize (Slapak et al., 2020).

4.7 Skin cancer (melanoma)

The MMP-1, MMP-2, and MMP-9 are associated in melanoma invasion and progression (Pereira et al., 2005). MMP-1 plays a role in melanoma invasion and is associated with the ability of melanoma cells to infiltrate surrounding tissues. Increased expression of MMP-1 is linked to a more aggressive form of melanoma. MMP-2 is implicated in melanoma progression and is associated with the invasive properties of melanoma cells. Elevated expression of MMP-2 is linked to a more aggressive form of melanoma. MMP-9 is also associated with melanoma invasion and disease progression. It contributes to the invasive behavior of melanoma cells. Increased expression of MMP-9 is linked to a more aggressive form of melanoma (Napoli et al., 2020).

4.8 Prostate cancer

Prostate cancer (PCa) represents nearly 20% of all new cancer diagnoses in the United States, with an estimated 164,000 (19%) new cases and over 29,000 (9%) related deaths reported (Siegel et al., 2018). It has been observed that patients lacking MMP-1 and MMP-9 expression in their prostate tumor cells and showing no TIMP-2 expression in normal glands experience a significantly shorter disease-free survival (DFS) (Roy et al., 2020). MMPs, specifically MMP-2 and MMP-9, have been associated with disease progression, invasion, and metastasis. MMP-2 is implicated in prostate cancer invasion and metastasis. It plays a role in degrading the ECM and facilitating the spread of prostate cancer cells to other parts of the body (Xie et al., 2016). MMP-9 is also associated with prostate cancer progression, invasion, and metastasis. It contributes to the invasive behavior of prostate cancer cells and their ability to spread to distant sites (Gong et al., 2014).

4.9 Glioblastoma (brain cancer)

MMPs and TIMPs play a crucial role in the progression of both primary and metastatic brain tumors, underscoring their significance. Several studies have provided compelling evidence of their involvement. The expression of various MMPs, including MMP-2, MMP-3, MMP-9, MMP-10, MMP-13, and MMP-14, has been linked to a more aggressive behavior in primary brain tumors such as glioblastoma, astrocytoma, and meningioma (Roy et al., 2020). This collective body of research highlights the pivotal role of MMPs and underscores their potential as important therapeutic targets in the context of brain tumor progression. MMP-2, MMP-3, and MMP-9 are implicated in glioblastoma invasion. MMP-2 increased angiogenesis in brain metastatic lesions, and MMP-9 are often found to be overexpressed in glioblastoma tumors, and their increased expression levels are associated with more aggressive disease and poorer patient outcomes (Hagemann, 2012).

4.10 Liver cancer

MMP-2, MMP-9, and MMP-7 have been associated with hepatocellular carcinoma progression. MMP-2 is known to be involved in hepatocellular carcinoma progression. Increased expression of MMP-2 is associated with the invasive behavior of HCC cells and the breakdown of the ECM, which allows cancer cells to infiltrate and metastasize. MMP-7 is also associated with HCC progression and is involved in the invasive and metastatic properties of HCC. The expression of MMP-7 is often elevated in HCC. MMP-9, like MMP-2, is implicated in the progression of HCC. It is associated with the invasive and metastatic potential of HCC cells. The upregulation of MMP-9 is linked to the aggressive behavior of the disease (Scheau et al., 2019).

4.11 Osteosarcoma

MMP-2, MMP-9, and MMP-14 are involved in osteosarcoma progression and metastasis. MMP-2 plays a role in degrading the ECM, which allows cancer cells to invade and migrate. Increased expression of MMP-2 is associated with a more aggressive form of osteosarcoma (Thanapprapasr et al., 2014). MMP-9 is implicated in osteosarcoma progression and metastasis. It contributes to the invasive and migratory properties of osteosarcoma cells. Elevated expression of MMP-9 is linked to a more aggressive disease (Bjørnland et al., 2005). MMP-14 is involved in the degradation of the ECM and facilitates cancer cell invasion. Upregulated expression of MMP-14 is connected to the ability of osteosarcoma to spread to other sites in the body. These MMPs' overexpression is associated with a poor prognosis for cancer and serves as a prognostic marker for osteosarcoma (Kunz et al., 2016).

4.12 Urinary bladder cancer

The activity of both MMP-2 and MMP-9 in urinary bladder cancer is significantly elevated in comparison with normal tissue, which is associated with tumor invasion and metastasis. In comparison of MMP-2 and MMP-9 in the tissues, it shows that MMP-9 is more involved in the growing and spreading of cancer cells in the bladder than MMP-2. MMP-2 is implicated in bladder cancer progression and is associated with the invasive behavior of bladder cancer cells. Increased expression of MMP-2 is linked to a more aggressive form of bladder cancer(-Vasala et al., 2003). The MMP-3 and -10 expressions in the bladder cancer tissues are also more as compared to normal tissue but the activity is less with respect to other tissues (Kudelski et al., 2023). Also, the other MMPs such as MMP-7 are specifically expressed in human bladder cancer (Szarvas et al., 2021). Finding high tissue expression of MMP14 is related with poor short-term prognosis in muscle-invasive bladder cancer. From the metaanalysis, it was conformed that the MMPs elevated expression leads to poor prognosis in bladder cancer. The metaanalysis confirmed that MMPs overexpression might be a prognostic factor predicting poor bladder cancer survival. The increased MMPs expression was correlated with poor outcome in bladder cancer.

5. Conclusions and future perspectives

MMPs played an important role in tumor progression as well as metastasis and recurrence. The clinical significance of MMPs as prognostic markers is evident in their pivotal roles throughout the cancer continuum. MMPs, including MMP-2 and MMP-9, play an integral part in cancer progression, particularly in tumor invasion and metastasis. Their heightened expression is associated with aggressive disease behavior, making them valuable indicators of prognosis and potential therapeutic targets. Understanding the relationship between diagnostic markers and MMPs is crucial, as both are linked to tumor progression, matrix remodeling, metastatic potential, and prognostic value. They contribute to different stages of the disease, from early detection to treatment decisions, assessment of the risk of metastasis, and ongoing patient monitoring. In summary, prognostic markers and MMPs represent two facets of cancer research that offer essential insights into patient outcomes and disease progression. Their impact is pervasive, spanning from the early detection of cancer to the management of metastatic disease, and their clinical significance continues to drive advancements in precision medicine and cancer therapy. Very few MMPs are clearly mentioned as diagnostic and prognostic cancer markers. Therefore, diligent introspection is strongly recommended focusing on identification of MMPs and establishing their relevance as diagnostic and prognostic cancer biomarkers of clinical utility.

The future prospects of MMPs as prognostic and diagnostic biomarkers in cancer hold great promise. With advancing technologies, including high-throughput omics techniques and sophisticated imaging modalities, MMPs can be analyzed in detail at various levels, from gene expression to protein activity. MMPs have shown their potential as biomarkers in different cancer types due to their involvement in tumor invasion, angiogenesis, and metastasis. By understanding the specific MMP profiles associated with different cancers, clinicians can develop targeted diagnostic assays to detect MMP expression patterns in patient samples. These assays can aid in early cancer detection, predict disease progression, and monitor treatment responses. Moreover, the development of innovative imaging techniques targeting MMP activity in vivo can provide valuable insights into tumor behavior and aid in personalized treatment strategies. As our knowledge of MMP biology expands and technologies become more sophisticated, MMPs are poised to play a crucial role in revolutionizing cancer prognostics and diagnostics, ultimately leading to improved patient outcomes and personalized therapeutic interventions.

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CHAPTER

14

From enzyme to predictors: Serine proteases in cancer diagnosis and prognosis

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1. Introduction

Cancer remains a significant global health challenge due to its increasing occurrence and mortality rates. Detecting cancer early and accurately predicting its behavior are vital for improving patient outcomes and creating tailored treatment plans. However, traditional markers often lack the necessary precision. Therefore, there is a pressing demand for novel and trustworthy biomarkers that can significantly enhance the effectiveness of cancer diagnosis, prognosis, and treatment monitoring.

Serine proteases are a significant class of enzymes that derive their name from the serine amino acid in their active site. They function primarily as endoproteases, breaking peptide bonds within polypeptide chains through hydrolysis. Their nucleophilic activity is enhanced by a catalytic triad of specific amino acid residues, allowing them to perform their essential role in protein modification and processing. This nucleophilicity is enhanced by a specific arrangement of three amino acid residues within the enzyme, known as the "catalytic triad." The catalytic triad includes three amino acids: aspartic acid 102 (Asp 102), histidine 57 (His 57), and serine 197 (Ser 197) (Di Cera, 2009). These three residues work together to create an optimal environment for the nucleophilic attack by serine on the peptide bond, thus facilitating the catalytic activity of serine proteases. This triad is also known as the charge relay system (Blow et al., 1969). This triad is essential for effective enzyme activity. During catalysis, histidine activates serine by removing a proton from its OH group, increasing its reactivity. Aspartic acid stabilizes the charged side chain of histidine, ensuring proper conformation. Proteases employ a sequential mechanism, generating multiple intermediates

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in the process. Notably, trypsin, subtilisin, prolyl oligopeptidase, and ClpP peptidase use an identical Asp-His-Ser configuration despite distinct protein folds. This triad enables them to catalyze peptide bond hydrolysis accurately. Overall, these insights into protease functioning highlight their pivotal role in various biological processes reliant on controlled protein degradation (Di Cera, 2009).

Serine proteases constitute a versatile class of enzymes with important roles across diverse biological processes. Notably, they manage vital processes including digestion, which breaks down dietary proteins in the digestive tract, and blood clotting, which is necessary for wound healing. Serine proteases are essential to immunological responses because they control immune cell activity and contribute to the complex complement system, which supports defense against infections. Moreover, these proteases engage in proteolytic networks, remodeling the extracellular matrix for tissue development and repair, while also influencing the signaling of growth factors and cytokines that regulate cell behavior.

Serine proteases have a significant role in the development of cancer. They promote tumor–stromal interactions in the cancer microenvironment, which has an impact on immune evasion, metastasis, and angiogenesis. These proteases perform a dual function, either promoting angiogenesis, a process that aids in the growth of tumors, or inhibiting tumors by interfering with immune responses and signaling pathways. Their potential as therapeutic targets are highlighted by the complexity of their actions in cancer. To effectively treat illnesses such as cancer and improve biological functions, it is important to understand the complex roles played by serine proteases. This knowledge provides insights into the physiology of disease and potential therapeutic paths (Tagirasa and Yoo, 2022).

In recent times, serine proteases have gained attention as valuable biomarkers for various diseases. Among these emerging candidates, serine proteases, for instance, were identified in the serum of cancer patients (Röthlisberger and Cortes, 2010). An example within this family is KLK2/hK2, a protein that serves as a biomarker for both breast and prostate cancer (Partin et al., 1999).

The body relies on a regulatory mechanism to control and inhibit the activity of proteases. This regulatory process is crucial for maintaining the proper functioning of various physiological processes. One key player in this regulation is the serine protease inhibitor, also known as serpin. A serpin is a type of protein that serves to manage and limit the activity of serine proteases. The balance between protease activity and inhibition is essential for preventing harmful effects that could arise from excessive protease activity. Dysregulation of serpins in humans has been frequently associated with a range of pathological conditions. These conditions include inflammation, cardiovascular diseases, cancer, and neurological disorders (Wolf et al., 1999).

Serpins have become a significant focus in scientific research, particularly as a potential therapeutic approach for cancer treatment. One role that serpins play is that of a tumor suppressor protein. A specific serpin called maspin is encoded by the SERPINB5 gene. Maspin is found in normal breast and prostate epithelium (cellular lining) and exhibits properties that inhibit tumor progression. It has been discovered that maspin effectively inhibits the invasion and growth of tumors, as well as the movement (motility) of human mammary tumor cells (Zhang et al., 1997).

Herein, we intend to discuss the function of serine proteases, their potential role as a biomarker for diagnosis of different cancers, recent advances of serine proteases in prognosis of cancer, and therapeutic uses of serine proteases.

2. Serine protease and cancer

Serine proteases play a significant role in regulating cellular processes by breaking down protein substrates through cleaving peptide bonds. Serine proteases are particularly abundant in humans, and their functions include initiating proteolysis, which has implications for various biological activities within the cell. Serine proteases play a critical role by cleaving protease-activated receptors (PARs), which are a class of G protein—coupled receptors that are present on a range of different cells. This cleavage initiates signaling cascades with far-reaching effects on physiological processes such as inflammation, clotting, migration, and immune responses (Heuberger and Schuepbach, 2019). Additionally, serine proteases have diverse roles in blood coagulation, digestion, apoptosis, fertilization, and immunity regulation, underscoring their significance in maintaining the proper functioning of various cellular processes and overall health.

The human genome harbors a variety of serine proteases, categorized into clans and families based on structural characteristics. They activate inactive proteins by catalytically cleaving peptide bonds, initiating their functional activation. Apart from their catalytic function, serine proteases engage in cell signaling pathways, affecting the activation or inactivation of downstream molecules. This intricate involvement underscores their importance in regulating cellular processes. Serine proteases are essential players in both normal physiological functions and disease mechanisms, demonstrating their significance in maintaining human health and offering potential therapeutic targets.

Matriptases, a type of membrane bound S1 proteases from the PA clan, show elevated gene expression in several cancers. Likewise, kallikreins, a large family primarily recognized for its involvement in blood pressure regulation via the kinin system, has also been linked in research as a biomarker for various cancers (Ramsay, 2008).

The human genome contains 10 peptidases from the SB clan, with nine belonging to the S8 family and one to the S53 family. Tripeptidyl-peptidase I (TPP-I) is the sole S53 family member, responsible for breaking down tripeptides. Proprotein convertase subtilisin-like kexin type 9 (PCSK9) is a significant protein controlling low-density lipoprotein (LDL) receptor levels in the liver and bloodstream. Mutations elevating PCSK9 activity relate to reduced cholesterol levels and an increased risk of coronary heart disease. Inhibition of PCSK9 offers a potential approach to lower LDL levels and mitigate heart disease risk (Page and Di Cera, 2008).

A glycoprotein belonging to the clan SR called lactoferrin has the ability to specifically target particular proteins from dangerous microorganisms such as *Haemophilus influenzae* and *Escherichia coli*. This proteolytic activity may interfere with how these proteins function, strengthening the body's defenses against infections brought on by certain viruses. This explains more about serine protease's role in the human body.

The relationship between serine proteases and cancer is described as a "complex interplay." This means that it is not a straightforward interaction; rather, it involves intricate dynamics where serine proteases interact with various factors in the tumor microenvironment and within cancer cells themselves. This complicated interplay involving serine proteases and cancer includes several areas of tumor biology and the tumor microenvironment. Serine proteases play an important part in the complex environment of tumor growth by helping cancer cells infiltrate surrounding tissues, form new blood vessels, and regulate signaling pathways within the tumor microenvironment shown in Fig. 14.1. Serine proteases are also involved in immune regulation within the tumor microenvironment shown in the figure. Alterations in the expression or activity of serine proteases can serve as diagnostic markers. Elevated levels of specific proteases in blood or tissue samples may indicate the presence of cancer or its severity. They can modulate the activity of immune cells, influencing whether the immune system detects and attacks cancer cells or tolerates their presence. Knowing how serine proteases affect cancer may help researchers develop new therapeutic approaches, diagnostic tools, and prognostic markers.

Serine proteases are involved in various functions such as blood clotting, activation of complement protein, inflammatory response, healing of wounds, and cleaving peptide bond.

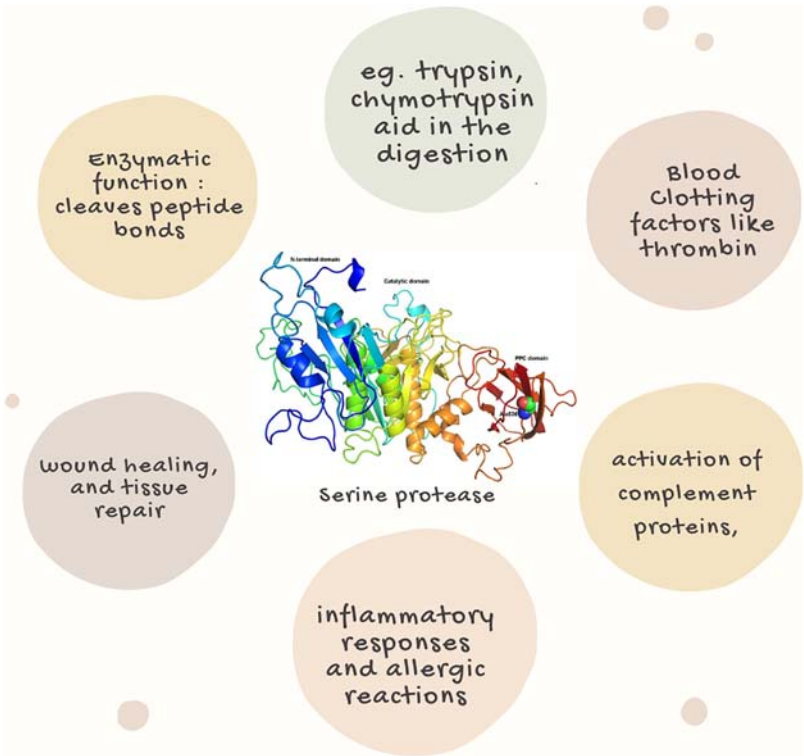

FIGURE 14.1 Serine proteases and their role in human health. Serine proteases play a very crucial role in different biological processes.

2.1 Extracellular matrix remodeling and tumor invasion

The capacity of a tumor to break out from its boundaries and infect adjacent tissues is the first stage in its fight for domination. Serine proteases such as urokinase-type plasminogen activator (UPA), tissue plasminogen activator (tPA), and plasmin seem to be key participants in this approach. These proteases allow tumor cells to move and invade neighboring tissues by catalyzing the breakdown of extracellular matrix components. Their masterstroke is the stimulation of plasminogen, a precursor protein that then activates matrix metalloproteinases (MMPs). MMPs that have been released cause devastation to the extracellular matrix, causing tumor cells to become more invasive (Angelucci and Alesse, 2012). Hence by cleaving ECM components, serine proteases facilitate cancer cell migration, invasion into surrounding tissues, and dissemination to distant sites.

2.2 Angiogenesis and metastasis

Serine proteases contribute to angiogenesis, the process of forming new blood vessels. This is crucial for supplying nutrients and oxygen to growing tumors. Serine proteases perform a new function in this chapter, supporting tumor microenvironment alteration. These proteases initiate a cascade of events that release proangiogenic factors by expertly cleaving vascular basement membrane components. Serine proteases, such as plasmin, can activate vascular endothelial growth factor (VEGF) by releasing it from the extracellular matrix. VEGF stimulates the proliferation and migration of endothelial cells, leading to the formation of new blood vessels. Transforming growth factor-beta (TGF-), hepatocyte growth factor (HGF), epidermal growth factor (EGF), and placental growth factor (PlGF) are other proangiogenic factors that are readily simulated by serine proteases. The activation, release, and regulation of these factors by serine proteases contribute to the angiogenic switch that supports tumor growth and progression. This molecular dance promotes the formation of new blood vessels, promoting neovascularization, which is necessary for tumor survival and ultimate spread (Brooks, 1996). Serine proteases play a direct role in promoting cell migration and invasion by cleaving adhesion molecules and ECM components. By degrading barriers and promoting cellular movement, proteases facilitate the spread of tumor cells within tissues.

2.3 Manipulation of signaling pathways

As the intricate workings of cancer unfold, serine proteases find their way to yet another spotlight: signaling pathway manipulation. These proteases use their enzymatic prowess to exert control on signaling molecules found in the tumor microenvironment, including growth factors and cytokines. Consider the tremendous effect of thrombin, a serine protease. It initiates a carefully choreographed interaction with protease-activated receptors (PARs), triggering a sequence of subsequent signaling events. Serine proteases can cleave and activate cytokines and chemokines, influencing immune responses and inflammatory signaling. For instance, proteases such as plasmin can process cytokines such as interleukin-1 beta (IL-1β), converting them to their active forms and amplifying inflammation in the tumor microenvironment. Serine proteases can also modulate the availability and activity of growth factors involved in signaling pathways. For instance, proteases such as plasmin can cleave

and activate latent growth factors, such as transforming growth factor-beta (TGF-β), releasing them from their inactive forms and promoting their signaling functions. This sophisticated performance fuels tumor development and supporting angiogenesis, revealing a new aspect of the cancer tale (Arora et al., 2007).

3. Serine protease inhibitor

Serine protease inhibitors or SERPINs are a class of proteins that play a critical role in regulating the activity of serine proteases, a group of enzymes that are involved in various essential physiological processes. SERPINs are characterized by their unique mechanism of action, which involves inhibiting the activity of serine proteases by forming tight and reversible complexes with them. This interaction between SERPINs and serine proteases is vital for maintaining the balance and control of proteolytic activities in the body.

While many serpins are known for inhibiting protease activity, some serpins do not possess this inhibitory function. Instead, they have diversified their roles to encompass a broad range of other important biological functions. Serpin dysregulation has been widely related to a variety of human illnesses. This includes diseases characterized by inflammation, cardiovascular issues, cancer, and neurological disorders.

Within the human genome, there are 37 different serpin proteins encoded by 10 different chromosomes. Among these serpins, 30 of them have the functional ability to act as inhibitors, serving an important role in regulating the activity of serine protease enzymes in various physiological processes (Mkaouar et al., 2019). There are two distinct categories of SERPINs: extracellular and intracellular. Extracellular SERPINs are encoded by genes on chromosome 14 and regulate protease activities involved in defending against pathogens, responding to injury, and controlling inflammation in the extracellular space. On the other hand, intracellular SERPINs, encoded by genes on chromosomes 6 and 18, regulate serine proteases within cellular compartments, contributing to various intracellular processes.

3.1 Role of serine protease inhibitors

Serpins are a diverse group of proteins that serve as key regulators in various physiological processes. SERPINs have a critical role in regulating blood clotting, a process known as the blood coagulation cascade. One example is antithrombin, a serpin that inhibits the activity of serine proteases involved in blood clot formation. By tightly controlling these proteases, antithrombin helps prevent excessive clotting and maintains the balance between clotting and bleeding.

SERPINs are involved in regulating tissue remodeling processes. Plasminogen activator inhibitor-1 and 2 (PAI-1 and PAI-2) are serpins that inhibit the activity of plasminogen activators. Plasminogen activators are enzymes that convert plasminogen into plasmin, which plays a crucial role in breaking down blood clots. By controlling these activators, PAI-1 and PAI-2 influence tissue repair and remodeling. Certain serpins, such as antitrypsin and antichymotrypsin, are involved in modulating the body's inflammatory response (Mkaouar

et al., 2019). They inhibit the activity of proteases that can contribute to tissue damage during inflammation, helping to regulate and mitigate the inflammatory process.

SERPINs also play roles in programmed cell death (apoptosis) and cell development. These functions are essential for maintaining proper cell growth, differentiation, and eliminating damaged or unwanted cells. SERPINs can influence the apoptotic pathway by interacting with and regulating certain proteases that are involved in apoptosis. These proteases, known as caspases, are responsible for executing the steps of apoptosis. By inhibiting specific caspases, SERPINs can control the timing and intensity of cell death, ensuring that apoptosis occurs in a controlled and appropriate manner.

Serpins in different situations also perform as hormone transporters, for example, SERPINA6 (corticosteroid-binding globulin) and SERPINA7 (thyroxine-binding globulin) are serpins responsible for binding and transporting hormones in the blood. SERPINA6 binds corticosteroids, which are involved in various physiological processes, including metabolism and immune responses. SERPINA7 binds thyroid hormones, which are crucial for regulating metabolism and overall bodily function (Mkaouar et al., 2019).

Serpins have been found to exhibit tumor suppression activity in humans. This means that these serpins have the ability to inhibit or hinder the development and progression of tumors. SERPINB5 (maspin) is believed to interfere with the signaling mechanisms that promote the rapid proliferation of cancer cells. By inhibiting these signaling pathways, the serpin can slow down or restrict the growth of cancer cells. Additionally, SERPINB5 (maspin) is thought to play a role in limiting the ability of cancer cells to spread to other parts of the body. It is associated with several cancers such as SERPINH1, also known as HSP47, which acts as a molecular chaperone. It assists in the proper folding of other proteins, ensuring their correct structure and function. This function is crucial for preventing the accumulation of misfolded proteins, which can lead to various diseases.

4. Serine proteases as diagnostic biomarkers

A diagnostic biomarker is a biological signal or clinical feature that assists in the diagnosis of a specific illness or condition in an individual patient. It determines whether or not a person is infected with the illness. A reliable diagnostic biomarker must be capable of detecting the illness properly and early on. Early identification is critical because it allows for timely intervention and enhances the likelihood of effective therapy. In cancer situations, early detection may result in more successful treatment measures and improved patient outcomes (Yousef and Diamandis, 2002).

Serine proteases have emerged as potential diagnostic biomarkers due to their involvement in various physiological and pathological processes. Altered expression and activity of specific serine proteases can serve as indicators of disease presence, severity, and progression. These proteases offer the advantage of disease-specific expression patterns, providing both specificity and sensitivity for certain conditions.

One significant application is early disease detection, as changes in serine protease levels often occur in the initial stages of diseases. This timely detection enhances the chances of

successful intervention and treatment. Moreover, serine proteases can differentiate between disease subtypes, aiding in precise diagnosis, particularly in diseases with multiple variants.

Their presence in bodily fluids facilitates minimally invasive testing, making diagnosis convenient for patients. Serine protease biomarkers can complement existing diagnostic methods, providing a comprehensive diagnostic approach. Additionally, these biomarkers can be incorporated into screening programs, contributing to early disease detection on a larger scale (Page and Di Cera, 2008).

By tracking changes in serine protease levels over time, disease progression can be monitored, enabling timely adjustments to treatment plans. The unique expression profiles of these proteases also contribute to personalized medicine by tailoring treatments to individual patients. Examples such as prostate-specific antigen (PSA) and tissue plasminogen activator (tPA) highlight their potential as diagnostic tools.

4.1 Using NIR probes for cancer-associated proteases detection as diagnostic biomarkers

Scott et al. (2021) used near-infrared (NIR) fluorescent probes for optical imaging systems that have been created to improve cancer knowledge and management. These probes aim to identify the activity of certain proteases, such as serine proteases, that are linked to cancer growth. The study focuses on two main NIR probe applications: early cancer diagnosis and personalized treatment evaluation. NIR probes have the potential to improve treatment results by addressing and detecting a higher level of serine proteases. They can also track serine protease activity throughout personalized therapy, offering useful insights into treatment efficacy and guiding treatment decisions.

4.2 ANX A2 as a serine protease in cancer invasion and metastasis as a diagnostic biomarker

ANX A2, an important member of the annexin protein family, is expressed in endothelial cells, macrophages, mononuclear cells, and cancer cells. It has a strong affinity for phospholipids and calcium. Its function as a serine protease is critical for endocytosis, exocytosis, autophagy, and cell-to-cell communication. Notably, ANX A2 coordinates the assembly of plasminogen and tissue plasminogen activator on cell surfaces, resulting in the conversion of plasminogen to plasmin.

In cancer biology, the serine protease activity of ANX A2 is required for the activation of prometalloproteases and latent growth factors, which are required for the degradation of the extracellular matrix and basement proteins during cancer cell invasion and metastasis. In human cancers, overexpression of ANX A2 is related to invasive behavior, aggressive tumors, treatment resistance, shorter disease-free life, and lower overall survival (Sharma, 2019).

4.3 Salivary serine proteases as biomarkers for oral squamous cell carcinoma diagnosis

According to Feng et al. (2019), the salivary protease profile of patients having oral squamous cell carcinoma (OSCC), oral benign tumors, chronic periodontitis, and healthy people

was studied. They concentrated on serine proteases utilizing human protease array kits, enzyme-linked immunosorbent tests, western blot, and immunofluorescence.

When compared with other groups, OSCC patients showed greater levels of certain serine proteases such as kallikrein 5. These proteases have been recognized as promising biomarkers for OSCC evaluation, and cutoff values were set. The combination of cathepsin V (cysteine protease), kallikrein 5, and ADAM9 (metalloprotease) showed the highest diagnostic effectiveness. Overall, examining the salivary protease profile, with a specific focus on the increased levels of cathepsin V, kallikrein 5, and ADAM9, holds potential for both screening and diagnosing purposes.

5. Serine proteases as prognostic biomarkers

A prognostic biomarker is a biological entity or clinical trait that provides predictive information about the progression of a disease or the likelihood of various health outcomes for an individual patient (Sechidis et al., 2018). An ideal biomarker should be capable of diagnosing the disease before it reaches the metastatic stage. In early-stage cancer, there is generally an 80% response rate to surgery and chemotherapy treatments (Muinao et al., 2018). Dysregulated expression of specific serine proteases has been linked to tumor growth, invasion, and metastasis, as well as other critical aspects of cancer biology. Researchers and doctors could obtain crucial insights into disease progression and patient survival by discovering and analyzing serine proteases as prognostic biomarkers in cancer patients.

Serine proteases have emerged as significant prognostic biomarkers, particularly in cancer. These enzymes play pivotal roles in disease progression, metastasis, and treatment response, rendering them valuable indicators of patient outcomes. Dysregulation of serine proteases is associated with aggressive tumor phenotypes and increased metastatic potential. Elevated expression of specific proteases often correlates with advanced disease stages and reduced overall survival, reflecting their prognostic significance. Different serine proteases and it's inhibitors are shown in Tables 14.1 and 14.2. These biomarkers also offer insights into treatment

TABLE 14.1 Serine proteases/serine protease receptors and their involvement in cancer.

Protease/Receptor	Cancers related	References
ANX A2	Liver cancer	Sharma (2019)
Kallikrein 5	Oral squamous cell carcinoma	Feng et al. (2019)
PAR-2 receptor	Ovarian, breast, and gastric cancer	Pawar et al. (2019)
Prostasin	Colorectal cancer	Selzer-Plon et al. (2009)
Matriptase	Ovarian cancer	Oberst (2023)
Urokinase-type plasminogen activator	Pancreatic, breast, and prostate cancer	Angelucci and Alesse (2012)
Fibroblast activation protein-alpha (FAPα)	Ovarian cancer	Scott et al. (2021)

TABLE 14.2 Serine protease inhibitors and their involvement in cancers.

Serine protease inhibitors	Cancers related	References
SERPING1	Prostate cancer	Peng et al. (2018)
Plasminogen activator inhibitor I and II	Breast cancer	Mkaouar et al. (2019)
SPINK1	Lung adenocarcinoma	Xu et al. (2018)
Hepatocyte growth factor activator inhibitor-1 (HAI-1)	Colorectal cancer	Selzer-Plon et al. (2009)
Maspin (SERPINB5)	Lung adenocarcinoma and bladder cancer	He et al. (2023), Mkaouar et al. (2019)
NEXIN-I	Colorectal cancer	Selzer-Plon et al. (2009)

response; serine protease levels can influence a tumor's reaction to therapies, aiding in treatment selection and customization.

Moreover, serine proteases provide information about metastatic risk, facilitating the stratification of patients into different prognostic groups. Monitoring these proteases can detect disease recurrence early, enabling timely intervention. Their presence in bodily fluids allows for noninvasive monitoring, improving patient comfort and facilitating routine assessment. Combining serine protease data with other clinical and molecular factors can lead to comprehensive prognostic indices that enhance predictive accuracy (Sechidis et al., 2018).

Examples of such proteases include members of the kallikrein family in prostate cancer, urokinase-type plasminogen activator (uPA) in breast cancer, and tissue factor pathway inhibitor-2 (TFPI-2) in various malignancies. By elucidating tumor aggressiveness, predicting metastatic potential, guiding treatment strategies, and aiding risk stratification, serine proteases hold promise as valuable tools for clinicians in making informed decisions for patient care. Their integration into clinical practice can lead to improved prognostic assessment, personalized treatments, and ultimately better patient outcomes.

5.1 As a prostate cancer prognosis biomarker

SERPING1, a serine proteinase inhibitor, is a potential biomarker for prostate cancer prognosis. In comparison with healthy prostate tissues, they discovered that SERPING1 expression was considerably reduced in prostate cancer tissues. Low SERPING1 expression correlated with higher Gleason scores, higher pathological grades, and advanced tumor stages. Kaplan−Meier analysis showed that lower SERPING1 mRNA levels predicted lower overall survival, lower disease-free survival, and lower biochemical recurrence-free survival. Multivariate analysis confirmed SERPING1's role as an independent prognostic marker for poor disease-free survival and biochemical recurrence-free survival (Peng et al., 2018).

5.2 SPINK1 as a serine protease inhibitor and its role in lung adenocarcinoma progression

SPINK1 is a serine protease inhibitor that ordinarily protects the pancreas from early enzyme activation, according to (Xu et al., 2018). In lung adenocarcinoma (LAC), however, SPINK1 alters its function and begins to promote cancer development, migration, and invasion. This impact is aided by increased synthesis of matrix metalloproteinase 12 (MMP12), which promotes cancer cell motility and invasion. Higher levels of SPINK1 in LAC tissues are related to worse survival, indicating that it might be used as a biomarker for disease prognosis.

5.3 Pericellular proteolysis and cancer progression: Roles of PAR-2 and serine proteases

Pericellular proteolysis aids tumor development by changing the extracellular matrix and boosting cell invasion, migration, and angiogenesis. Pericellular proteases interact with cells directly via protease-activated receptors (PARs), a subset of G protein−coupled receptors. In many malignancies, dysregulated trypsin-like serine proteases activate PAR-2, which is overexpressed in advanced tumors. Pawar et al. (2019) indicated that certain protease agonists can alter PAR-2 signaling. Furthermore, accumulating data shows that PAR-2 and membrane-anchored serine proteases are known to interact, potentially boosting tumor growth and metastasis. By targeting these pathways, novel mechanism-based techniques for treating advanced and metastatic malignancies can be developed.

5.4 Role of serine proteases and inhibitors in colorectal cancer: Implications for tumor suppression and malignant progression

Prostasin, a serine protease with tumor-suppressing properties, relies on its activation by matriptase, an oncogenic serine protease. In colorectal cancer, the mRNA levels of prostasin were found to be reduced in dysplasia and carcinomas compared with normal tissues from the same individuals. Conversely, the inhibitor of prostasin, protease nexin-1 (PN1), exhibited a significant increase in colorectal cancer tissues, as well as in dysplastic tissues when compared with normal tissues from the same individuals and healthy volunteers. Similarly, the two isoforms of hepatocyte growth factor activator inhibitor-1 (HAI-1A and HAI-1B) showed similar expression patterns (Selzer-Plon et al., 2009).

Immunohistochemical analysis revealed that prostasin predominantly localizes to the apical plasma membrane in normal colorectal tissue, while its polarization within colorectal cancer tissues exhibited considerable variation. These findings suggest that the upregulated levels of PN1 may play a role in inhibiting prostasin activity in colorectal cancer, potentially contributing to the cancer's progression. Nevertheless, further comprehensive studies are warranted to determine whether this dysregulation of prostasin by PN1 is a driving factor or a consequence of cancer development (Selzer-Plon et al., 2009).

5.5 Expression of matriptase and its inhibitor HAI-1 in epithelial ovarian cancer

Oberst (2023) investigated the expression of matriptase, a type II transmembrane serine protease, and its inhibitor, hepatocyte growth factor activator inhibitor-1 (HAI-1), in epithelial ovarian cancer, and explored potential clinicopathological correlations. Immunohistochemistry was used to assess the expression of matriptase and HAI-1 in 54 epithelial ovarian cancer samples. The results showed that 72% of the tumors tested were positive for matriptase, while 20% were positive for HAI-1. Interestingly, all HAI-1-positive tumors also expressed matriptase.

Advanced-stage ovarian tumors (III/IV) displayed reduced expression of matriptase, and HAI-1 compared with early-stage tumors (I/II). In stage I/II tumors, matriptase and HAI-1 were expressed in higher percentages, with some tumors coexpressing both proteins. However, stage III/IV tumors showed lower matriptase expression, and only one tumor coexpressed HAI-1. These findings suggest that an imbalance in the matriptase: HAI-1 ratio may play a crucial role in the development of advanced ovarian cancer.

5.6 Maspin (SERPINB5) in lung adenocarcinoma: A prognostic biomarker and therapeutic target

He et al. (2023) investigated the effect of clade B serpins (SERPINBs), which are serine protease inhibitors, in cancer, especially lung adenocarcinoma (LUAD). Initially thought to be a member of the tumor suppressor gene family, certain SERPINBs were shown to have activities other than blocking protease activity. After conducting a thorough review of multiple cancer databases and transcriptome data, it was observed that SERPINB5 is upregulated and demethylated in LUAD and that unusually high levels of expression are linked to poor overall survival. Furthermore, SERPINB5 knockdown in LUAD cells decreased cell proliferation, migration, and epithelial—mesenchymal transition (EMT), whereas SERPINB5 overexpression increased cell proliferation, migration, and invasion. According to these results, SERPINB5 may be exploited as a biomarker for cancer prediction and a therapeutic target.

Tables 14.1 and 14.2 summarize different serine protease/receptors and inhibitors and cancer associated with it.

6. Serine proteases in therapeutics

Serpins, or serine protease inhibitors, are crucial regulators of numerous biological processes, accounting for 2%—10% of proteins found in circulating plasma. These versatile inhibitors are involved in a myriad of biological responses, including but not limited to, coagulation regulation, neurotrophic factor modulation, hormone transportation, inflammation and complement control, angiogenesis, and blood pressure regulation.

Certain serpins have been linked to the progression or regression of specific cancers, highlighting their potential as therapeutic or diagnostic tools. For instance, plasminogen activator inhibitor-1 (PAI-1), a key regulator of thrombolysis, can either inhibit or promote tumor growth. However, recent studies have shown that blocking PAI-1 can decrease cancer cell

migration, proliferation, and survival by altering the function of the urokinase-type plasminogen activator receptor.

Maspin, a noninhibitory serpin, enhances the sensitivity of cancer cells to apoptosis and curbs their migration, thereby acting as a tumor growth inhibitor. Pigment epithelium-derived factor (PEDF) is another serpin with potent antiangiogenesis activity that also induces apoptosis in cancer cells (Zheng et al., 2013).

7. Conclusion

In conclusion, this comprehensive exploration of the intricate roles of serine proteases and their inhibitors in various aspects of cancer biology sheds light on their significant potential as diagnostic biomarkers and therapeutic targets. The studies reviewed herein collectively underscore the pivotal roles played by these proteases in cancer invasion, metastasis, tumor progression, and overall patient prognosis.

The integration of near-infrared (NIR) fluorescent probes into cancer research, as highlighted by Scott et al., represents a remarkable advancement in the field of cancer diagnostics and personalized treatment evaluation. These probes offer a window into the activity of cancer-associated proteases, such as ANX A2, which are instrumental in promoting cancer growth and metastasis. The identification of ANX A2's serine protease activity as a diagnostic biomarker provides a promising avenue for improving patient outcomes and tailoring therapies more effectively.

Furthermore, the exploration of serine protease inhibitors, exemplified by SPINK1, PROSTASIN, and SERPINBs, opens up new avenues for understanding the delicate balance between protease activation and inhibition. The divergent functions of these inhibitors in promoting or suppressing cancer progression highlight the complexity of their roles in different cancer contexts. Leveraging these findings could provide opportunities for novel therapeutic interventions and prognostic assessments in specific cancer types, as evidenced in lung adenocarcinoma and colorectal cancer research. The emergence of salivary serine proteases as potential diagnostic biomarkers in oral squamous cell carcinoma underscores the potential of noninvasive approaches for cancer screening and diagnosis. The identification of specific protease profiles, such as kallikrein 5 and ADAM9, offers a tangible framework for enhancing both the sensitivity and specificity of oral cancer detection.

The association between pericellular proteolysis, protease-activated receptors (PARs), and cancer progression unveils a novel avenue for targeted therapeutic strategies. Disrupting the interaction between dysregulated serine proteases and PAR-2 could hold promise in mitigating advanced tumor growth and metastasis, offering innovative prospects for managing aggressive malignancies.

In summary, this chapter collectively demonstrates the intricate roles of serine proteases and their inhibitors in cancer biology. Their multifaceted contributions to cancer invasion, progression, and patient prognosis underscore their potential as diagnostic biomarkers and therapeutic targets. This synthesis of research findings not only enhances our understanding of cancer mechanisms but also paves the way for the development of more precise diagnostic tools and tailored therapeutic interventions to ultimately improve patient outcomes in the battle against cancer.

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CHAPTER

15

Cathepsins as diagnostic and prognostic markers in oral cancer

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1. Introduction

Oral squamous cell carcinoma is the most common type of cancer affecting approximately 90% of oral cavity malignancies. Globally, it ranks as the sixth most prevalent cancer (Scully and Bagan, 2009). In India, it is classified as the third most frequent form of cancer and has become a concerning issue due to the prevalent use of tobacco products both in smoked and smokeless forms (Warnakulasuriya, 2009).

The forecast for the progression of this condition is often difficult due to its aggressive nature, with more than 60% of cases showing cervical lymph node metastasis upon initial diagnosis. The survival rate decreases significantly in OSCC patients with cervical lymph node metastasis (LNM) (almost by 50%) and there is a higher likelihood of distant metastasis. Therefore, prediction of clinical outcomes heavily relies on the presence or absence of LNM (Massano et al., 2006). Currently, prognosis assessment and treatment decisions are primarily based on tumor node metastasis (TNM) staging system. However, it has been highlighted that relying solely on TNM may not be accurate since even early-stage tumors like T1 and T2 can exhibit LNM and aggressive behavior leading to mortality as well (Woolgar et al., 1995).

Researchers have investigated various clinical and pathological factors to identify patients with a greater likelihood of developing lymph node metastasis. These indicators aid in determining the need for personalized interventions, such as prophylactic elective neck dissections. Histological markers, including tumor differentiation, thickness, invasion pattern, depth of invasion, presence of lymphovascular emboli or perineural invasion, and occurrence of regional lymph node metastasis or extracapsular spread in the lymph nodes, have also been linked to prognosis in this regard. Furthermore, there have been numerous molecular studies investigating biomarkers that could potentially predict prognostic outcomes in oral

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squamous cell carcinoma. However, the majority of these studies have not yielded conclusive findings (Massano et al., 2006). As a result, there is still ambiguity and insufficient evidence regarding their use in routine clinical practice. Therefore, there is an ongoing search for a more reliable and consistent parameter for prognosis. A thorough grasp of OSCC's molecular mechanism is crucial for early identification and effective treatment to enhance patient survival (Woolgar et al., 1995).

Tumor growth, invasion, angiogenesis, and metastasis are all processes in cancer progression that can be initiated by cathepsins. Cathepsins play a crucial role in degrading and processing various substances such as growth factors, hormones, and cell adhesion proteins (Rakash, 2012).

2. Proteases and cathepsins

Proteases are enzymes found across various cells and are recognized for their vital role in several critical biological functions in healthy cells. In cancerous or transformed cells, these proteases have been shown to significantly contribute to tumor growth and advancement at both primary and metastatic locations. Additionally, research suggests that proteases may not only be produced solely by cancer cells but also prompt nearby stromal cells to generate proteolytic enzymes, potentially leading to further tumor expansion (Rakash, 2012; Decock et al., 2005).

Additionally, invasion and metastasis were originally thought to be late events in cancer development involving proteases. Nevertheless, studies have indicated that these processes can also take place in the initial phases. Moreover, different phases of cancer advancement encompass protease-related mechanisms such as increased cell proliferation, decreased apoptosis, participation of white blood cells (WBC's), enhancement of angiogenesis, and induction of multidrug resistance (Koblinski et al., 2000; López-Otín and Bond, 2008).

The complete array of proteases has been categorized as the degradome. The human protease family is composed of at least 569 proteases and homologs divided into five catalytic classes: 194 metallo, 176 serine, 150 cysteine, 28 threonine, and 21 aspartic proteases; however, not all of them have been associated with cancer (Decock et al., 2005; Koblinski et al., 2000; López-Otín and Bond, 2008).

Lysosomes, being cellular organelles with an acidic interior, contain various enzymes such as proteases and glycosidases that aid in cellular catabolism. Among these enzymes are cathepsins, a group of lysosomal proteases with diverse functions. The term "cathepsin," which originates from the Greek term "kathepsein" that means "to digest," primarily represented the proteases found in the endosomal/lysosomal proteolytic system and active in a slightly acidic environment. The concept of "catheptic activity" was first observed in gastric juice during the 1920s. Cathepsins are classified into three families of proteases: serine (including cathepsins A and G), aspartic (including cathepsin D and E), and 11 cysteine cathepsins (including cathepsins B, C, F, H, K, L, O, S, V, X, and W) (Reiser et al., 2010; Rudzińska et al., 2019; Khaket et al., 2019).

The different ways in which cathepsin is created are very similar, but they vary in their designs, methods of action, and the proteins that they break down. The triggering and

processing of proteases present in the lysosomes are closely connected to various factors within the organelle's environment. These factors include pH levels, proteases involved in processing, glycosyl side chains of membrane proteins as well as the composition of the lipids of membranes of the organelles. Cathepsins can be activated through two different pathways. The first pathway involves self-activation, where glycosaminoglycans and negatively charged surfaces aid in self-stimulation of cysteine cathepsins. Alternatively, there is another method that involves activation by other proteases. For example, cathepsin C and X are activated by cathepsin L or S to remove their prodomains, while cathepsin D cleaves and activates cathepsin B (Khaket et al., 2019; Chen et al., 2017; Jiang et al., 2023).

Cathepsins, which are predominantly found in lysosomes and thrive in an acidic environment, can exhibit diverse localization depending on different circumstances. They have the ability to be transported to the nucleus where they modify histones and bring about regulation of expression of genes, or they can be released into the extracellular matrix from the cell surface for various functions. In malignant cells, cathepsins relocate to the cell surface to break down the extracellular matrix (ECM), thereby promoting tumor cell invasion and metastasis. A selection of cathepsins, such as cathepsin B and L, has also been observed entering the bloodstream and identified in serum. However, the levels of these cathepsins are known to undergo significant fluctuations, which could potentially make them useful clinical indicators (Khaket et al., 2019; Chen et al., 2017).

The unique physiological roles of cathepsins are partly attributed to their varied distribution within and outside the cells. An acidic environment, like that found in lysosomes, is essential for optimal functioning of the lysosomal cathepsins. Among the cathepsins, cysteine cathepsins, are most frequently found in all tissue types and are functionally diverse. Their precise and targeted proteolytic activity allows them to participate in numerous activities like antigen processing and activation of proteolytic enzymes. Moreover, they can be involved in the remodeling of the ECM during healing of wounds and tumor invasion (Chen et al., 2017; Jiang et al., 2023; Conus and Simon, 2008; Dhalla and Chakraborti, 2014).

In recent years, researchers have discovered that cathepsins, originally known for their digestive function, play a crucial role in diverse normal and abnormal activities. These enzymes are involved in development, differentiation, angiogenesis, as well as reproductive processes such as sperm and ovum occurrence. Additionally, cathepsins contribute to organism apoptosis and immune responses while also playing a role in skeletal metabolism (Conus and Simon, 2008; Dhalla and Chakraborti, 2014).

The functions of cathepsins span a broad spectrum, occurring within cells and outside of them. Recent advancements in genomics, proteomics, imaging technology, as well as thorough analysis of knockout and transgenic mice have significantly improved our understanding of cathepsin function. These studies have shown that cathepsins play a vital role in modifying specific substrates, rather than just being redundant enzymes involved in protein turnover. As a consequence, cathepsins have diverse roles such as antigen presentation in the immune system, remodeling of collagen, and processing neuropeptides and hormones. Cathepsins have been implicated in various conditions including atherosclerosis, chronic kidney disease, pulmonary fibrosis, neuroinflammation, osteoarthritis, rheumatoid arthritis tuberculosis, and different types of cancer (Rudzińska et al., 2019; Khaket et al., 2019; Jiang et al., 2023).

The functions of cathepsins both inside and outside cells distinguish them from other types of proteases, such as metalloproteases or serine proteases. This unique ability to act extracellularly plays a role in cancer cell invasion and spreading to distant tissues through nearby tissues, blood vessels, and lymph vessels. Consequently, targeting cathepsins has potential implications for anticancer treatment (Rakash, 2012; Jiang et al., 2023).

The cysteine and aspartic groups include the most extensively researched cathepsins in cancer, specifically cathepsins B, L, and D. These enzymes undergo changes after being synthesized and move to specialized compartments within cells called lysosomal vesicles. In certain types of cancer cells, they can either be released from the cell or become attached to the cell membrane. Their functions are regulated by naturally occurring inhibitors such as cystatins A, B, and C (Decock et al., 2005; Koblinski et al., 2000; López-Otín and Bond, 2008).

In recent years, there has been significant research on cathepsin inhibitors. These inhibitors have been thoroughly investigated since the discovery of the first crystal structure of cathepsins. They work by binding to specific groups in the active site of cathepsins, effectively reducing or blocking their function without causing damage to the enzymes themselves. Depending on how they bind, these inhibitors can be classified as reversible or irreversible and further categorized as either endogenous (such as thyroxine, precursor peptide, serpin family) or synthetic varieties (Rakash, 2012; Decock et al., 2005; Koblinski et al., 2000).

3. Cathepsins in cancer

Cathepsins play a central role in cancer progression by enabling the breakdown of ECM components and promoting cancer cell migration and invasion. Additionally, they are implicated in tumor growth, angiogenesis, and resistance to treatment. Abnormal expression and oversecretion of cathepsins are associated with their involvement in these processes (Rakash, 2012; Reiser et al., 2010; Rudzińska et al., 2019).

Cancer cells overexpress cathepsins, leading to increased invasion and spread of cancer. Cathepsins are involved in breaking down and restructuring the extracellular matrix within the tumor microenvironment, contributing to enhanced tumor advancement and infiltration. Higher levels of cathepsin generally correspond with a worse prognosis (Reiser et al., 2010; Rudzińska et al., 2019; Jiang et al., 2023).

Higher concentrations of extracellular cathepsins have been observed in various cancer types, including breast cancer, colon carcinomas, cancers of the lung and pancreas, skin-related malignancies, carcinomas of prostrate and bladder, as well as head and neck carcinomas. The rise in levels is frequently linked to heightened activity, which can be ascribed to the stimulation of cytokines associated with tumors. As a result of this process, cell—cell adhesion molecules are shed and enzymatically cleaved, causing disruption in cellular contact and promoting metastasis. For example, the adhesion molecule E-cadherin, which has a tumor suppressive role in the epithelial cells, is cleaved by external cathepsins B, L, and S. This promotes the invasion of tumors into surrounding tissues (Reiser et al., 2010; Rudzińska et al., 2019; Jiang et al., 2023).

Cathepsins found outside of cells contribute to the advancement of cancer by participating in "shedding, a process where proteases release the extracellular portions of proteins on to the cell surface" (López-Otín and Bond, 2008; Reiser et al., 2010). This process transforms proteins that are attached to the cell membrane into soluble forms, leading to decreased expression on the surface of the cells. Influence on subsequent physiological processes is exerted by cathepsins. In addition to influencing the shedding of various cell adhesion molecules, these enzymes also impact the Ras signaling pathway. This pathway is an important intracellular signaling pathway involved in cancer progression (Reiser et al., 2010; Rudzińska et al., 2019).

Research has indicated a connection between cathepsin B and cancer. It has been demonstrated that cathepsin B can lead to destruction of the basal lamina and remodeling of the connective tissue facilitating tumor growth and progression (Reiser et al., 2010; Rudzińska et al., 2019; Khaket et al., 2019).

The involvement of cathepsin K in the remodeling/modification of the ECM has been observed. Its expression has been found to be associated with malignancies in breast and prostate cancers, leading to a considerable reduction in cancer metastasis by inhibiting this specific enzyme. The primary contribution of cathepsin K lies in its potent collagenolytic activities, as it primarily aids in breaking down extracellular components and collagen. Recent research suggests that cathepsin K may also have implications for the invasive behavior of cancer cells. It is believed to play a crucial role in promoting increased motility of oral squamous cell carcinoma cells through direct association with protein degradation processes (Reiser et al., 2010; Rudzińska et al., 2019; Khaket et al., 2019).

Cathepsins play a significant role in the degradation and reconstruction of the ECM. They are can activate the prourokinase-type plasminogen activator and other proteases, which enhances their impact on breaking down tumor matrix and basement membrane. Additionally, intracellular cathepsins have been shown to contribute to tumor progression by influencing processes that can have both protumorigenic and antitumorigenic effects. The breakdown of intracellular collagen is an example of how cathepsins can potentially promote intracellular protumor activity (López-Otín and Bond, 2008; Reiser et al., 2010; Rudzińska et al., 2019).

The pathophysiological role of cathepsins in the spread of cancer has generated interest in examining their potential as biomarkers for poor prognosis and development of metastasis in various malignancies. Elevated levels of several cathepsins (V, B, and D) have been linked to distant metastasis and poorer disease-specific survival rates in breast cancer as well as in melanomas, lung carcinomas, and oral carcinomas. Additionally, increased expression of cathepsin K has been observed in breast carcinoma, invasive variant, with higher protein levels associated with an increased likelihood of metastasis. Remarkably, a significant increase in cathepsin K expression was found in the stroma surrounding lung carcinomas. This discovery suggests that production of CTSK by the stromal cells may potentially facilitate or control tumor cell invasion (Koblinski et al., 2000; López-Otín and Bond, 2008; Reiser et al., 2010).

Cathepsins S and L present in the extracellular location predispose the liberation of epidermal growth factor receptor (EGFR) and plexins, which in turn can activate the Ras pathway. Likewise, extracellular cathepsin D has been shown to break down ECM proteins, leading to the release of growth factors such as fibroblast growth factor. These factors that promote growth are important in breaking down the basement membrane, promoting cell

movement, and spreading tumors through both self-stimulation and nearby signaling mechanisms (Reiser et al., 2010; Rudzińska et al., 2019; Khaket et al., 2019).

The use of cathepsin inhibitors has shown promise in preventing the invasion and metastasis of cancer cells. Furthermore, combination therapies that involve cathepsin inhibition are being increasingly studied as potential anticancer treatments. For example, conjugating cathepsin inhibitors with radiotherapy or chemotherapy, as well as using clinical inhibitors originally developed for osteoporosis treatment, could potentially help reduce cancer progression (Khaket et al., 2019).

4. Cathepsins in the progression and prognosis of oral cancer

Oral squamous cell carcinoma is a commonly occurring and extremely lethal form of cancer in the mouth. Despite utilizing different treatment methods such as chemotherapy, radiotherapy, and surgery, there has not been a significant improvement in mortality rates. Multiple cathepsins have been extensively researched and linked to oral squamous cell carcinoma (Jiang et al., 2023; Leusink et al., 2018; Yadati et al., 2020; Vigneswaran et al., 2000). Fig. 15.1.

Fig. 15.1 demonstrates an overview of role of cathepsins in oral cancer.

4.1 Cathepsin B

Cathepsin B has been involved in tumor evolution, proliferation, and development of metastasis, and cellular invasion has been identified. It plays a major part in the degradation

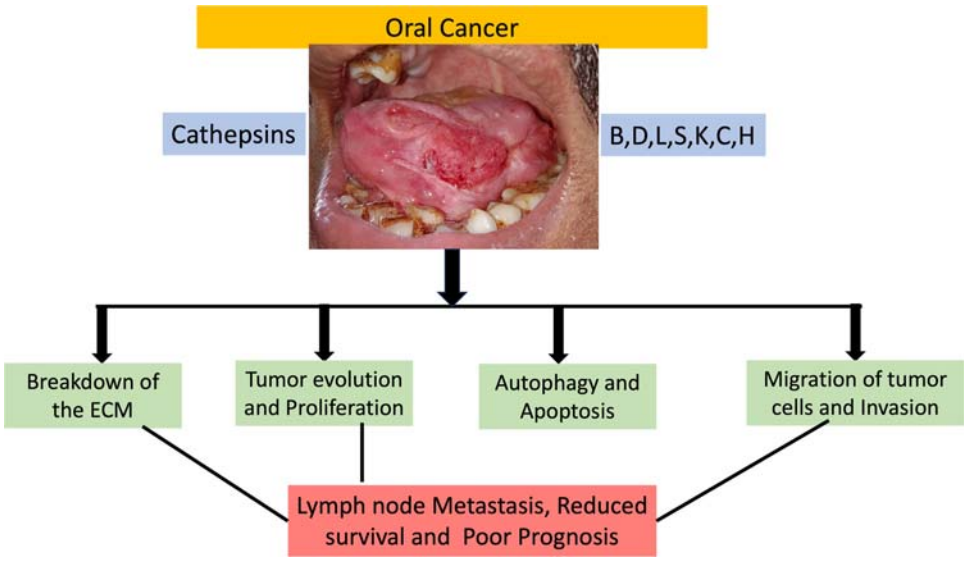

FIGURE 15.1 Role of cathepsins in the prognosis of oral cancer. *Adapted From Jiang et al. (2023).*

of basement membranes and extracellular matrix. During this process, cathepsin B may liaise with caspase and annexin II tetramer before being localized to strategic places where proteolytic activation of the ECM takes place. As a result, cathepsin B has the potential to initiate a series of proteolytic reactions by activating plasminogen, matrix metalloproteinases, and urokinase plasminogen activator. This process may also influence the viability of cancer cells. Cathepsin B impedes TLR3-mediated apoptosis making the cancer cells resistant to programmed cell death (Gandour-Edwards et al., 1999). According to a study by Pakfetrat et al. (2022), the cathepsin B along with Cav-1 gene expression was found to be increased in OSCC tissues. This higher expression has been associated with greater severity and aggressiveness of OSCC. The researchers also discovered that when they specifically targeted cathepsin B mRNA using ribozymes, there was a decrease in its mRNA, protein, and activity levels in oral cancer cells. Additionally, it was noted that there was reduction in the motility and invasiveness of these cells. This confirmed that cathepsin B is directly involved in the progression and invasive capacity of oral cancer but also proposed a potential strategy for controlling its advancement by targeting it with mRNA inhibitors.

In a study conducted by Yang et al. (2016), it was observed that patients with lymph node metastasis and poorly differentiated tumors showed an increased expression of cathepsin B. The researchers also discovered a significant correlation between cathepsin B expression and lower overall survival rate among the oral carcinoma patients. Additionally, through immunohistochemical analysis of tissues from 280 OSCC (oral squamous cell carcinoma) patients, the investigators confirmed the involvement of CTSB expression in OSCC by demonstrating its association with lymph node metastasis and higher tumor grade. Furthermore, the study suggested that CTSB expression is associated with reduced survival in OSCC patients, highlighting its potential as a biomarker for predicting prognosis specifically within Taiwanese individuals.

Vigneswaran et al. (2000) showed that oral squamous cell carcinoma exhibits heightened levels of cathepsin B and cathepsin D, along with altered distribution patterns. It is noteworthy that a considerable number of tumors displaying elevated cathepsin B also demonstrated concurrent overexpression of cathepsin D, which could have important implications for the biological processes. They said that cathepsin D facilitates the processing of procathepsin B both intracellularly and extracellularly. The expression of cathepsin B and cathepsin D was limited to suprabasal keratinocytes in skin and oral mucosa. However, in SCC, there is an overexpression of CB and CD, which was observed in the peripheral cells of the tumor islands while it was negative of weak expression in the central parts of the tumor islands composed of more differentiated cells. These findings suggest a distinct mechanism regulating the cathepsin expression in tumorous keratinocytes as compared with normal cells. Elevated levels of cathepsin B in oral carcinomas were found to be strongly associated with advanced tumor stage and poor histologic grade. Additionally, increased expression of cathepsin D was significantly associated with presence of metastasis and a high proliferative activity. These findings suggest a notable connection between the expression levels of both cathepsins B and D in oral carcinomas and their patterns of local invasion as well as metastatic growth. Therefore, these proteases hold great potential as valuable prognostic markers and viable targets for gene therapy in treating oral cancer.

In a study conducted by Yang et al. (2010), it was found that there is an increased presence of cathepsin B protein associated with cellular transformation. The expression of cathepsin B

was examined in three different cell lines as well as in clinical samples of OSCC patients and their adjacent normal epithelia. Cancer cells (HB56 and HB96) exhibited increased levels of cathepsin B protein and mRNA using Western blot analysis and real-time PCR. Moreover, studies using immunohistochemistry and real-time PCR have shown that cathepsin B protein and mRNA levels are also elevated in tumor tissues compared with adjacent normal epithelia in OSCC patients. Thus, expression of cathepsin B augments with advancement of OSCC, both within laboratory settings and living organisms. This indicates its potential as a biomarker for OSCC.

Shabbir et al. (2022) found that individuals diagnosed with OSCC showed greater levels of cathepsin B in their saliva compared with healthy individuals. This finding indicates that cathepsin B has the potential to be a valuable biomarker for diagnosing and monitoring OSCC across different grades, potentially leading to enhanced survival rates and prognosis.

4.2 Cathepsin D

Upregulation of cathepsin D has been strongly associated with the spread of tumors, lower levels of tumor aggressiveness as determined by cellular characteristics and higher rates of cell proliferation. Tumors can release procathepsin B, which gets stimulated by cathepsin D. This activation triggers the prourokinase plasminogen activator and may also result in ECM modification through a cascade of other enzymes. These mechanisms potentially contribute significantly to the progression of cancer by facilitating the invasion and spreading of malignant cells (Mijanovic et al., 2021). According to Vigneswaran et al. (2000), cathepsin B and D levels are increased in oral squamous cell carcinoma. Higher levels of these proteins may impact biological aggressiveness, metastatic potential, histological malignancy, and proliferative activity. As a result, assessing cathepsins B and D levels could serve as diagnostic and prognostic markers for oral cancer.

Previous studies conducted on HNSCC reveal that cathepsin D may have a potential role in predicting cervical lymph node metastasis (Gandour-Edwards et al., 1999). Immunohistochemical analysis by Marsigliante et al. (1994) revealed an increase in cathepsin D levels, specifically in the peripheral parts of tumor islands. This increase was consistently higher as compared surrounding fibroblasts in the stroma, the inflammatory cells, as well as normal epithelium. These findings were in concordance with that of Zeillinger et al. (1992), who also observed increased cathepsin D levels in both carcinomas at the primary site and in the metastatic lymph nodes from these group of patients.

In a study conducted by Gandour-Edwards et al. (1999), they found that immunohistochemical analysis showed cathepsin D expression in 38% of patients diagnosed with T1 or T2 primary HNSCC. Interestingly, the expression levels for cathepsin D varied within the tumor and exhibited a significant increase in areas of keratinization. A noteworthy finding was the presence of concentrated and intense positivity in specific tumor nests at the invasive margins. Tumors expressing cathepsin D were found to have nearly double the likelihood of being related with LNM, indicating that it could potentially serve as an independent prognostic marker for cervical LNM. This discovery calls for additional research and exploration into this area.

4.3 Cathepsin L

Nakashima et al. (2012) reported that elevated production of cathepsin L, both in protein and mRNA levels, is commonly observed in precancerous oral lesions. The increased quantities of cathepsin L may thus be implicated in the beginning of development of oral cancer. They suggested that enhanced cathepsin L is linked with LNM and a poor prognosis in oral squamous cell carcinoma, indicating its potential as a marker for prediction of prognosis. The precise mechanism by which cathepsin L contributes to the advancement of oral cancer remains uncertain. However, research has shown that increased cathepsin L expression in squamous cell carcinoma cells can enable local invasion and metastasis through degradation of basement membrane and ECM.

Nagaraj et al. (2006) found that the expression and activity of cathepsin B and L increased significantly in OSCC cells, which could potentially enhance the invasion of these cells. Cathepsin inhibitors have shown potential in preventing cell invasion and inhibiting matrix disintegration, thus highlighting the significant role of these proteases in cancer cell invasion among smokers. Inhibiting cathepsins B and L could be a promising approach to inhibit the dissemination and development of metastasis of smoking-associated oral cancer (Jiang et al., 2023).

Macabeo-Ong et al. (2003) observed that nonprogressive oral dysplasia exhibited significantly lower levels of cathepsin L mRNA and protein compared with oral cancer. However, the difference in cathepsin L levels between progressive dysplasia and oral cancer was not significant. These findings suggest that an overexpression of cathepsin L may be indicative of the potential progression from dysplasia to advanced stages of oral cancer. Additionally, an association has been found between increased levels of cathepsin L and the occurrence of lymph node metastasis as well as a negative prognosis in patients with oral cancer. These results indicate that cathepsin L shows potential as a prognostic marker in this context.

4.4 Cathepsin S

Da Costa et al. (2020) said that cathepsin S may have a significant impact on the progression of tumors. This is achieved through several mechanisms, including promotion of apoptosis and autophagy, interference of angiogenesis, and obstructing the passage and infiltration of malignant cells. Cathepsin S can induce apoptosis through two pathways: an intrinsic pathway involving death of mitochondria and an extrinsic pathway facilitated by death receptors.

Previous studies have found that certain pathways, such as PI3K, AKT, mTOR, and JNK, can be activated by reactive oxygen species to induce autophagy and trigger mitochondria apoptosis when cathepsin S is blocked. Furthermore, the p38 MAPK/JNK signaling pathway may be involved in regulating autophagy and apoptosis in human oral cancer cell lines. Additionally, cathepsin S has been shown to facilitate neurogenic inflammation and pain through the cleavage of protease-activated receptor 2. Within the microenvironment of oral cancer, there are both presence and activity of cathepsin S (Hsieh et al., 2017; Chen et al., 2018).

4.5 Cathepsin K

Cathepsin K plays a crucial role in OSCC invasion, migration, adhesion, and protein degradation (Imad et al., 2023; Yamashita et al., 2017). According to a study by Bitu et al. (2013), it was found that the presence of CTSK was observed in both cells of the tumor and the stroma using immunohistochemistry. A significant observation from the study revealed that strong expression of CTSK in stromal cells may have a protective effect, as weak expression correlated with poorer prognosis specifically in early-stage tumors within the tumor microenvironment. The findings suggest that the expression level of CTSK in either tumor or stromal cells could potentially affect prognosis outcomes. They also found that silencing cathepsin K in HSC-3 tumor cells resulted in a decrease in invasion. This finding indicates that the prognosis could vary depending on the expression of CTSK in either the tumor or stromal cells. In aggressive tongue HSC-3 cells, a decreased activity and expression levels resulted in diminished invasion when tested in vitro. However, the level of cathepsin K found within carcinoma cells did not show any correlation with patient outcomes related to cancer. This implicates that probably cathepsin K in the stroma could have a protective role in tongue cancer progression.

Leusink et al. (2018) analyzed the expression of cathepsin K in OSCC and its relationship to clinicopathological variables, specifically lymph node metastasis. They found that lower levels of SERPINB13 inhibitor for CTSK were associated with LNM and a negative prognosis in oral and oropharyngeal SCC. Increased CTSK expression was linked with lymph node metastasis and worse prognosis in OSCC. The protein expression of CTSK can serve as a valuable tool in diagnosing cT1-T2 N0 OSCC, with a high negative predictive value (89%). However, further validation in a larger prospective cohort study is necessary.

The researchers suggested two possible reasons for the inverse correlation between patient survival and cathepsin K expression in OTSCC stroma. “One possibility is that decreased cathepsin K expression results in a decrease in growth factor activity. Another possibility is that increased cathepsin K activity occurs in areas where it is needed to compensate for the absence of other enzymes.” As enzymatic activity increases within the stroma, there may be a feedback mechanism involving suppression of active cathepsin K, such as cystatin activation. Additionally, it is possible that several overexpressed enzymes at the tumor edge unintentionally interfere with the maturation process of procathepsin K. In vivo settings are inherently more complex than laboratory studies, and factors such as growth signals and feedback mechanisms play a crucial role in determining overall prognosis, especially when considering metastasis and tumor growth potential (Leusink et al., 2018; Seo et al., 2023).

Yamashita et al. (2017) discovered that the expression of CatK varied among different types of oral squamous cell carcinomas, including SAS, OSC20, HSC2, HSC3, HSC4, and Ca9-22. Interestingly, despite the varying levels of cathepsin K expression within cells themselves, no release into the extracellular space was observed. The researchers found that overexpression of cathepsin K hindered the proliferation of HSC3 cells; however, it promoted their migration and invasion. On the other hand, inhibiting cathepsin K with a specific inhibitor had contrasting effects on OSCCs. Furthermore, the study revealed that increased expression of cathepsin K promotes adhesion and regulates invasion and migration in oral

squamous cell carcinomas. The results highlight that overexpression of cathepsin K contributes to enhanced invasion and migration, while inhibiting cathepsin K has the opposite effect.

4.6 Cathepsin C and H

In the study by Magister et al. (2015), Cathepsin C, an enzyme found in cytotoxic T lymphocytes and NK cells, plays a crucial role in activating granzymes A and B. The release of perforin and activated granzymes into the synaptic region between killer cells and their targets is part of the process known as granule exocytosis. Among these molecules, granzyme B is particularly effective at inducing apoptosis in targeted cells. Initially produced as a progranzyme B with inhibitory dipeptide elements, it becomes activated by cathepsin C within secretory lysosomes. Although alternative processes for its activation have been proposed, cathepsin C primarily facilitates the activity activation of granzyme B.

Various cathepsins (B, D, L, S, K, C, and H) play key roles in the tumor evolution and progression of oral cancer. Specifically, the cathepsins have a role in the destruction of the ECM, enhancing the motility and invasive capacity of the cancer cells, tumor growth and proliferation, participating in autophagy and apoptosis, etc. Thus, the cathepsins predispose to enhanced lymph node metastasis and reduced survival and may be responsible for the poor prognosis seen in oral cancer (Jiang et al., 2023).

5. Functions of cathepsins in the treatment of oral cancer

Focusing on cathepsins as a potential management modality for OSCC could offer a fresh perspective, complementing established methods such as chemotherapy, radiotherapy, and surgery.

The function of cathepsin B in the spread and invasion of OSCC is significant. By utilizing RNA inhibitors, the progression and metastasis of this enzyme can be effectively controlled. To improve gene therapy for OSCC, the targeting of cathepsin B and D shows promising potential. The plant-derived compound platyphyllenone has shown potential as an antimetastatic agent. It works by reducing the phosphorylation of p38, increasing E-cadherin expression, and decreasing cathepsin L expression to achieve its antimetastatic effects. Further research is needed to fully understand the exact mechanism, but it appears that reducing cathepsin L levels could have important therapeutic inferences for highly metastatic OSCC by potentially impacting epithelial-to-mesenchymal transition.

In addition, inhibiting the activation of cathepsin S in cancer cells has been shown to increase levels of microtubule-associated protein 1 light chain 3 protein. This results in the conversion of cytoplasmic LC3-I into lapidated LC3-II. A study by Chen et al. highlighted that sulforaphane, a natural compound found in cruciferous plants, can suppress cathepsin S and LC3 activities, thereby reducing oral cancer cell migration. These findings propose an innovative approach for treating OSCC using sulforaphane (Jiang et al., 2023; Yadati et al., 2020).

6. Conclusion

Cathepsins are a diverse group of enzymes that play various roles in both intra- and extracellular settings. In oral cancer, cathepsins degrade extracellular matrix proteins to accelerate cancer cell invasion and metastasis. These enzymes might play a crucial role in tumorigenesis and could serve as potential targets for the development of drugs to treat oral cancers. The prominent focus on the extracellular function of cathepsins has garnered significant interest in the biomedical realm. These proteins have emerged as valuable diagnostic indicators and potential targets for therapeutic interventions in cancer and other disorders. The predictive significance of cathepsins in oral cancer remains unclear, and further research is needed to understand the various pathways linking cathepsins to oral cancer. Currently, the use of cathepsin-based treatments for oral cancers is still speculative. However, gaining a deeper understanding of human cathepsin biology will enable future scientists to develop more effective treatments for a wide range of diseases involving cathepsins.

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CHAPTER

16

Role of kallikreins in prostate cancer

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Abbreviations

AR Androgen receptor
CD3 Cluster of differentiation 3
CRPC Castration-resistant prostate cancer
EMT Endothelial-mesenchymaltransition
ERK Extracellular signal regulated kinase
IGF Insulin growth factor
KLK Kallikrein
PAI-1 Plasminogen activation inhibitor-1
PAR Protease activated receptor
PCa Prostate cancer
PI3Kβ Phosphoinositide 3-kinase β
PSA Prostate specific antigen
PThRP Parathyroid hormone-related protein
shRNA Small hairpin RNA
uPA Urokinase-type plasminogen activator
uPAR Urokinase-type plasminogen activator receptor

1. Introduction

Prostate cancer (PCa) is regarded as the second most prevailing type of cancers observed among male population in Western countries. Nearly all patients with localized PCa may survive for 5 years or more. Nonetheless, majority of the cancer-related deaths are caused by recurring and/or metastatic PCa, which is currently incurable (Pascale et al., 2017). The

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primary therapeutic approaches for advanced PCa center on the androgen dependence of the cancer cells. These approaches involve blocking the androgen receptor (AR) axis to prevent the generation of androgens through the use of the compounds such as degarelix and abiraterone, as well as by directly targeting the AR with drugs such as enzalutamide and bicalutamide (Crona and Whang, 2017). Thus far, it has been shown that these tactics are effective in considerably raising the chances of survival for PCa patients. Furthermore, because the cancer cells could gradually acclimate to the androgen-targeting therapy rendering it nonresponsive, these patients in the course of time develop castrate-resistant prostate cancer (CRPC), which ultimately results in metastasis (Crona and Whang, 2017). Understanding the molecular mechanisms in PCa cell progression during androgen targeting therapeutics (ATT) is essential to resolving this problem and may result in the creation of novel therapeutic drugs that aid in averting disease recurrence.

It is widely recognized that proteases have a functional role in the advancement of cancer by either stimulating or inhibiting tumor growth (López-Otín and Matrisian, 2007; Vasiljeva et al., 2019). Kallikreins belong to a family of 15 secreted serine proteinases and are well known for the onset and spread of cancer (Filippou et al., 2016; Kryza et al., 2016). KLKs are serine proteases that resemble trypsin or chymotrypsin and have a molecular mass of 25−30 kDa. KLKs are released in a variety of body fluids and expressed in the glandular epithelia of numerous organs (Ando et al., 2021). They play significant roles in various cancer types, including prostate cancer, and have a broad spectrum of substrates (Innocenti, 2018).

Certain KLKs, such as KLK2, KLK3, KLK4, and KLK14, are recognized to be essential in various stages of the development of prostate cancer. These stages include encouraging cell invasiveness, stimulating tumor growth, facilitating the transition from epithelial to mesenchymal tissue, degrading extracellular matrix, and causing bone metastases (Avgeris et al., 2012).

Because KLKs could exhibit a critical part in the pathobiology of prostate cancer, scientists and medical professionals have been tempted to examine KLK expression and activity as both a valuable biomarker and a potential therapeutic target in a number of clinical trials. The main points of this review are the roles played by KLK2, KLK3, KLK4, and KLK14 in prostate cancer. Furthermore, several subheadings discuss the involvement of other KLK family members in the development of prostate cancer.

2. KLK14

Due to its potential connection in multiple types of cancer, KLK14 is the subject of several research groups. The exact function of KLK14 in the body is unknown. KLK14 is expressed in the skin, where it may have a role in the physiology of the epidermis and trigger the primary effects on skin desquamation. In vitro, desmoglein-1 protein can be directly degraded by KLK5 and KLK7 (Furio et al., 2014, 2015; Yoon et al., 2007). Although an increase in KLK14 level has been observed in prostatic and normal breast tissues, its exact role in those tissues is still unknown (Hooper et al., 2001; Kryza et al., 2016; Rajapakse and Takahashi, 2007)

In prostate tissues, KLK14 expression has been shown to be dysregulated. Prostate-specific antigen (PSA/KLK3) was shown to cause biochemical relapse, and that has been found to be linked to high KLK14 expression (Borgoño et al., 2007; Lose et al., 2012; Lintula et al., 2005). Genetic studies have shown polymorphisms in the KLK14 gene, which are strongly correlated with PCa fierceness (Lose et al., 2012; Rose et al., 2018), and could corroborate the relationship of KLK14 with PCa. It has been discovered that persons who develop CRPC also have high expression levels of KLK14. This shows that KLK14 expression is upregulated in metastatic prostate cancer and is reactivated in PCa cells that avoid ATT, indicating that serum or PCa tumor KLK14 expression levels may be helpful in predicting the aggressiveness of prostate malignancies or the efficacy of treatment (Kryza et al., 2020).

KLK14's location at the cell lamellipodia, which is crucial for regulating cellular motility, provides the first indication of the protein's function in PCa cells (Innocenti, 2018). An increase in the proteolytic activity of KLK14 has resulted in the creation of a tubular network made up of cohesive cells, altering the cell pattern. Notably, rather than spreading as a single cell, PCa cells can infiltrate neighboring tissues by forming a coherent cell network (Nagle and Cress, 2011). Thus, evidence supporting KLK14's role in regulating PCa cell migration comes from migration experiments conducted utilizing KLK14-overexpressing or -mutant PCa cells as well as KLK14-selective inhibitors (Kryza et al., 2020).

The proteolytic action of KLK14 on a number of recognized substrates, including laminin-subunit proteins LAMA5, LAMB2, LAMC1, KLK3/PSA, KLK14, as well as a number of additional suspected substrates, was validated by cell-based proteomics profiling. The majority of KLK14 substrates that have been uncovered have been linked to the progression of PCa in the past and are associated in regulating cell adhesion, migration, and morphology. For instance, it has been noted that aggressive PCa is marked by DSG-2, a protein implicated in desmosome production (Barber et al., 2014). Two other KLK14 substrates, IGSF8 and SEMA3C control androgen-independent PCa cell proliferation and metastasis in addition to their role in cellular morphology (Levina et al., 2015; Tam et al., 2017). Several more recently discovered substrates, including SMOC2, SDC4, and agrin, have been observed to play critical roles in the development of PCa (Shvab et al., 2016; Wang et al., 2015)

It is well known that KLK14 controls how other proteases, including ADAM10 and ST14/matriptase, function. Two KLK14-cleavage sites (Arg208 and Arg614) have been detected in ST14/matriptase, which are situated between its catalytic site and transmembrane domain. These findings suggest that KLK14 regulates matriptase shedding, a process that could be linked to PCa cell invasion (Cheng et al., 2013; Wu et al., 2020). Besides, matriptase has been observed to be one of the activators of pro-KLKs, which was detected in the epidermis (Sales et al., 2010). Therefore, its shedding via KLK14 may play a significant role in controlling the epidermal proteolytic pathway(s) that leads to skin desquamation.

Numerous genes that KLK14 was demonstrated to regulate have been connected to the development of PCa. For example, LCN2 has been associated with the development of CRPC and the regulation of PCa cell migration, invasion, and proliferation (Ding et al., 2015, 2016). Additionally, the neuroendocrine phenotype, CRPC, and a stem cell phenotype in PCa have all been connected to the expression of MDK and advanced PCa (Erdogan et al., 2017; Nordin et al., 2013). Because they alter KLK14-dependent transcriptome regulation, both the stimulation of the MAPK pathway and the attenuation of the AR axis are important in metastatic prostate cancer. The development of CRPC and decreased survival rates for PCa

patients are associated with MAPK activation, more specifically with the ERK/MEK pathway (Mukherjee et al., 2011; Rodríguez-Berriguete et al., 2012).

Even though PCa cells do not need androgens to grow and survive, stimulation of the AR axis is usually thought to be necessary for PCa development in spite of CRPC formation (Einstein et al., 2019). Prostate tumors do contain AR-negative subpopulations of PCa, albeit this is becoming more widely acknowledged. The absence of neuroendocrine characteristics and AR expression in some subpopulations of metastatic PCa cells has been shown recently. Remarkably, studies have demonstrated that these "double-negative" PCa cells have heightened susceptibility to MAPK inhibitors (Bluemn et al., 2017). In these conditions, KLK14 could be an important modulator. Clinical trials are currently highlighting the significance of trametinib's (an inhibitor of MEK1/2) activity in metastatic castration-resistant prostate cancer (NCT02881242).

A number of synthetic KLK inhibitors were developed upon altering naturally occurring protease inhibitors (Di Paolo et al., 2019). A bioengineered form of the SunFlower Trypsin Inhibitor (SFTIWCIR) with a Ki of 7 nM for KLK14 offers over 1000-fold selectivity against KLKs such as KLK5 and KLK7. Conceivably, SFTIWCIR is one of the most powerful and selective KLK14 inhibitors created in the recent past (de Veer et al., 2017). To enable the use of SFTI-based inhibitors in preclinical models, additional inhibitor optimization is necessary because these are small peptide compounds and have a relatively short serum half-life.

3. KLK2/KLK3/KLK4

3.1 KLK2

Prostate cancer that has progressed from normal to high-grade prostatic intraepithelial neoplasia (HGPIN) lesions is associated with a considerable overexpression of KLK2 on the mRNA as well as the protein levels (Darson et al., 1999). Prostate-specific antigen, or KLK3, has been shown to have a significantly higher ratio of KLK2: PSA mRNA, particularly in high-grade prostate cancer. This suggests that changes in the KLK2:PSA ratio, which has been demonstrated in tumor tissues, and KLK2 overexpression are closely linked to carcinogenesis and the progression of prostate neoplasia. The ratio can be used as an efficacious aid to revamp to improve prostate cancer diagnosis, grading, and prognosis (Emami and Diamandis, 2008). Several studies have provided accurate descriptions of some of the potential molecular processes that could explain why KLK2 overexpression is relevant in prostate cancer.

In spite of the presence of antiandrogens, KLK2 associates with ARA70, an AR coactivator that promotes AR transactivation and preservation of AR activity (Shang et al., 2014). These results imply that KLK2 may be important for the maintenance of CRPC. According to the activation of its several substrates, KLK2 was found to exert a critical role in prostate cancer progression by promoting cell proliferation, tumor cell invasion, and metastases (Fig. 16.1). By cleaving IGF-binding proteins and raising IGF availability in the prostate microenvironment, KLK2 can activate the IGF-IGFR-dependent mechanisms (Réhault et al., 2001).

KLK2, KLK3, KLK4, and KLK14 have key roles in prostate cancer progression, as demonstrated by several mechanisms.

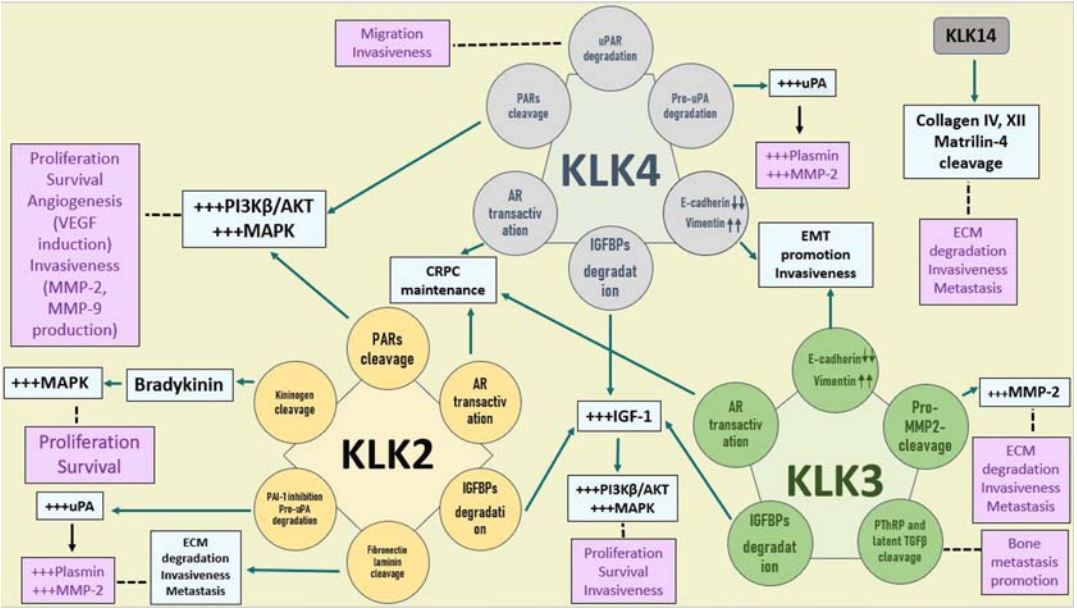

FIGURE 16.1 Prostate cancer progression involves KLK2, KLK3, KLK4, and KLK14. KLK2, KLK3, and KLK4 are efficient enzymes that release (+++) IGF-1, a key growth factor in cancer formation, by hydrolyzing its regulating IGFBP. IGFs increase cancer cell proliferation, survival, and migration by activating the PI3Kβ/AKT and MAPK signaling pathways (+++). Bradykinin, an agonist of the B2 receptor, is released from kininogen via KLK2 and activates the MAPK pathway (+++), promoting prostate cancer cell proliferation and survival. KLK2 and KLK4 degrade ECM proteins indirectly by cleaving pro-uPA, which accumulates (+++) and stimulates the uPA–uPAR axis. The uPA–uPAR axis stimulates plasmin and MMPs, which promote tumor cell invasion and metastasis. KLK2 inhibits the covalent uPA inhibitor protein PAI-1, facilitating uPA–uPAR system–related proteolysis. KLK4 directly cleaves uPAR, affecting cell adhesion, migration, and invasion. KLK2 and KLK4 cleave PAR1 (KLK4) and PAR2 (KLK2 and KLK4), activating the PI3Kβ/AKT and MAPK pathways. PARs can lead to enhanced invasiveness, survival, IL-6, IL-8, and VEGF expression, as well as amplification of MMP-2 and MMP-9 production. KLK4 and KLK3 may modulate EMT during prostate cancer progression by reducing E-cadherin levels and increasing vimentin levels. KLK2, KLK3, and KLK14 can degrade ECM through fibronectin and laminin cleavage, pro-MMP-2 cleavage, and cleavage of collagen IV, XII, and matrilin-4. KLK3 may promote bone metastases through PThRP and latent TGF-β cleavage. KLK2, KLK3, and KLK4 can trigger AR transactivation, suggesting their potential role in CRPC maintenance.

Protease-activated receptor 2 (PAR2) is cleaved by KLK2, which increases DU145 and PC3 cell proliferation in vitro (Mize et al., 2008). Several reports showed their participation in an increase in cells protrusion and survival, promotion of IL-6, IL-8, and VEGF expression, and stimulation of MMP-2 generation. Black et al. (2007), Liu et al. (2006), and Wilson et al. (2004) have supported the potential role of PARs, a member of the GPCR family of receptors, in the autocrine and paracrine pathways of prostate cancer. However, the exact biochemical mechanism that links the increase in the proliferation of prostate cancer cells and PAR2 activation by KLK2 is currently unknown. Moreover, KLK2 has the ability to break fibronectin and laminin directly, which permits penetration of the blood vessels and surrounding connective tissues.

Additionally, it was discovered that KLK2 indirectly degrades ECM proteins by cleaving prourokinase-type plasminogen activator (pro-uPA) and/or downregulating plasminogen activator inhibitor-1, an uPA inhibitor protein. This causes uPA to accumulate and then stimulates the uPA−uPA receptor (uPAR) axis (Avgeris et al., 2012; Mavridis et al., 2014). After that, the plasmin and MMPs in the tumor microenvironment are activated by the uPA-uPAR axis, which promotes tumor cell invasion and the release of different growth factors from the extracellular matrix (Avgeris et al., 2012; Mavridis et al., 2014).

3.2 KLK3

KLK3 (aka PSA) is the generally used biomarker for diagnosis, prognosis, and prediction for prostate cancer (Diamandis, 2010). Remarkably, prostate cancer is associated with downregulation of KLK3 mRNA and protein. Prostate cancer progression may benefit from KLK3's potential role, as evidenced by the high amounts of this protein found in the peritumoral fluid surrounding primary and metastatic prostate tumors (Swedberg et al., 2010). KLK3 is activated in response to androgens (Lawrence et al., 2010), just as KLK2, and it can also increase AR activity in prostate cancer cells (Saxena et al., 2012). In fact, KLK3 can typically interact with ARA70, thereby promoting AR transactivation (Niu et al., 2008), which plays a pivotal role in the reduction of both the mRNA and protein levels of AR in prostate cancer cells transfected stable with short hairpin RNA (shRNA) against KLK3.

The important roles that KLK3 plays in the carcinogenesis of prostate cancer are as follows: (1) it activates the IGF–IGFR axis, which stimulates mitogenic and antiapoptotic stimuli in the cells; (2) it activates MMP-2 from its precursor (pro-MMP-2), promoting ECM degradation and the intrusiveness of prostate cancer cells (Pezzato et al., 2004); (3) it induces EMT-like alterations that changes in vitro via decrease in E-cadherin and an increase in vimentin, respectively, albeit the precise molecular mechanisms of action are currently unknown (Veveris-Lowe et al., 2005); and (4) it promotes the formation of osteoblastic metastasis, conceivably via parathyroid hormone associated protein and the cleavage of the inactive form of TGF-β, which induces bone resorption and bone formation (Mavridis et al., 2014). However, several investigations revealed that KLK3 could elicit paradoxical effects, either stimulating or attenuating the development of cancer, based on its levels, tissue settings, and stages of prostate cancer.

3.3 KLK4

It has been demonstrated that KLK4 mRNA is an independent biomarker for distinguishing between benign and malignant prostatic lesions, which has been associated with advanced stages of illness (Avgeris et al., 2011). Similar to KLK2 and KLK3, androgens regulate KLK4 hormonally. AR and KLK4 are part of a well-defined positive feedback loop (Lawrence et al., 2010). More precisely, promyelocytic leukemia zinc finger protein is an inhibitor of AR and mTOR1, and KLK4 suppresses it to enhance AR and mTOR1-mediated mitogenic and antiapoptotic signaling (Jin et al., 2013). Furthermore, a complex process involving PAR1, IL-6, and extracellular signal−regulated kinase (ERK) regulated pathways play an important role in KLK4-mediated AR transactivation (Réhault et al., 2001).

Several in vitro studies have raised the possibility that KLK4 may have additional modes of action that promote prostate cancer. KLK4 may have an effect on EMT modulation as prostate cancer advances (Mavridis et al., 2014; Veveris-Lowe et al., 2005). Overexpression of KLK4 has been shown to upregulate vimentin expression and inhibit E-cadherin in PC3 cells. It has been demonstrated that KLKs enhance in vitro PC3 cell motility and support spindle-shaped morphology (Einstein et al., 2019). Prostate cancer KLK4 has been proposed to facilitate bone metastases (Mavridis et al., 2014; Veveris-Lowe et al., 2005).

By activating and/or regulating the uPA−uPAR axis, KLK4 can significantly accelerate the invasion of prostate tumors. Additionally, by cleaving IGF-binding proteins and overstimulating IGF-IGFR signaling, KLK4 can trigger mitogenic and antiapoptotic stimuli (Réhault et al., 2001). Furthermore, KLK4 cleaves PAR1 and PAR2, which promotes the growth of prostate cancer cells. Specifically, it has been noted that PAR1 cleavage can cause cell proliferation and Src (sarcoma) kinases to become phosphorylated after ERK 1/2 activation (Mavridis et al., 2014; Ramsay et al., 2008).

4. Other KLK-related peptidases

4.1 KLK1

It is currently unclear whether KLK1 expression and/or activity are altered in prostate cancer. But KLK1, a kininogenase-containing protein, stimulates angiogenesis by generating kinin and activating kinin B12 and B2 receptors, which raises the possibility that it plays a role in the growth of prostate cancer cells (Réhault et al., 2001; Biyashev et al., 2006). It has been shown recently that KLK1 treatment of DU145 cells significantly enhances their migration and invasion by activating PAR1 (Gao et al., 2010).

4.2 KLK5 and KLK7

Skin-related disorders are known to be impacted by KLK5 and KLK7 (Kryza et al., 2016). When compared with normal prostatic tissues, these kallikreins were observed to be decreased in prostate cancer (Mavridis et al., 2014; Réhault et al., 2001; Swedberg et al., 2010). The pathobiology of prostate cancer has not yet been determined, despite recent research pointing to a potential role for these KLKs as activators of tumorigenic processes or predictive biomarkers for CRPC (Mo et al., 2010).

4.3 KLK6

Neurodegenerative illnesses have been observed to be the primary domain in which KLK6 is engaged (Kryza et al., 2016). When compared with normal tissues, it was found to be attenuated in a number of metastatic cancers, including prostate cancer (Lawrence et al., 2010). Prostate cancer has been demonstrated to be significantly increased when KLK6 methylation is elevated; nevertheless, more investigation is required to determine the precise pathways by which KLK6 contributes to the development and advancement of prostate cancer (Olkhov-Mitsel et al., 2012).

4.4 KLK11, KLK12, KLK14, KLK15

Uncertainty surrounds the physiological roles and pathological consequences of various KLKs. Nonetheless, certain clinical studies have demonstrated the predictive significance of KLK12, KLK14, and KLK15 in the development of prostate cancer. These are as follows: (1) a rare homozygous genotype for KLK12's rs3865443 single nucleotide polymorphism (SNP) has been linked to an increased risk of prostate cancer (Lose et al., 2013). Higher tumor grade, stage, and Gleason score have been observed to be linked to lower KLK11 mRNA levels (Nakamura et al., 2003); malignant versus benign samples exhibit KLK15 mRNA overexpression, which has been linked to advanced pathological stage in individuals with prostate cancer (Mavridis et al., 2013). However, further research is needed to gain a better understanding of the involvement of KLK11, KLK12, KLK14, and KLK15 in the pathobiology of prostate cancer.

5. Targeting KLK-related peptidases: A broad range of drugs

Targeting KLK-related peptidases is undoubtedly a viable treatment strategy for prostate cancer. There is an increasing interest in creating instruments to further limit (or restore normalcy to) KLK function (Sotiropoulou and Pampalakis, 2012). The ability of naturally occurring/endogenous inhibitors to regulate KLK under physiological settings has been well documented (Mavridis et al., 2014). Cations, such as Zn^{2+} levels (Mavridis et al., 2014), and proteinaceous inhibitors such as SERine protease inhibitors (serpins), Kazal, and Kunitz-type inhibitors, are the prominent examples of the endogenous repertoire for regulating KLK activity (Swedberg et al., 2010). Serpins attenuate KLKs via the irreversible spring-suicide mechanism that includes insertion of the inhibitors reactive loop in the active site. This irreversibly disrupt the catalytic triad, rearranging the structures of the inhibitor and protease, and inserting the inhibitor's reactive loop into the active site, serpins block KLKs. Conversely, inhibitors of the Kunitz and Kazal types block the activity of KLKs by means of the standard method of direct competition (Swedberg et al., 2010). They merely prevent substrate access by binding to the proteases' active sites in a lock-and-key manner. Naturally occurring inhibitors are quite potent, but their poor selectivity poses a significant challenge for any therapeutic application. Their broad range activity is compensated for in normal physiology by their ability to function in a temporal and spatial manner. However, since drugs are frequently administered through the bloodstream, therapeutic inhibition needs to be very selective and should have minimal off-target inhibition (Swedberg et al., 2010).

6. KLKs targeted agents in prostate cancer

Prostate cancer can be effectively treated with a few potential KLK-targeted therapeutic drugs, according to preclinical and clinical research. These medications are separated into four groups according to their mechanism of action. These are (1) KLK-engineered inhibitors;

(2) KLK-activated prodrugs; (3) KLK-targeted miRNAs, siRNAs, and shRNAs; (4) KLK vaccines and antibodies.

6.1 KLK-engineered inhibitors

6.1.1 MDKP67b

MDKP67b is a recombinant serpin-type inhibitor that targets human KLK2 and is used to treat prostate cancer. It was designed and evaluated using ACT, an endogenous inhibitor of α1-antichymotrypsin, as a bio-scaffold by Med Discovery (http://med-discovery). The most selective Lys-Leu-Arg-#-Thr-Thr sequence was used to construct this powerful and efficacious drug by replacing the ACT cleavable link and surrounding residues (Cloutier et al., 2004). It was demonstrated that MDPK67b substantially inhibited KLK2 compared with other unrelated serum proteases and could, in a dose-dependent manner, block PAR2 activation (Swedberg et al., 2010).

When given to experimental animals such as rodents and primates, MDPK67b was shown to elicit a rather benign toxicological profile, with no apparent immunogenicity problems, and no interference with blood coagulation-related proteases (Swedberg et al., 2010). MDPK67b safety, pharmacokinetic, and pharmacodynamic characteristics are being assessed in healthy volunteers in several clinical trials (http://med-discovery). Testing this inhibitor on asymptomatic, hormone-resistant prostate cancer patients with increasing PSA levels should be an important stage for evaluating its efficacy. However, because these patients experience chemotherapy-induced neutropenia, the effectiveness of MDPK67b's inhibition of KLK4, 5, and 14 and its induction in transgenic mice are in question (http://med-discovery). As such, the drug's specificity has not been conclusively proven.

6.1.2 Sunflower trypsin inhibitor (SFT-1)

The 14-cyclised amino acid peptide SFTI-1 (Lose et al., 2012; Swedberg et al., 2010) has been shown to strongly inhibit KLK4 activity. A KLK4 inhibitor with enhanced efficacy and selectivity over other proteases is produced by the remodeled SFTI-1, SFTI(FCQR) upon insertion of the tetrapeptide, Phe-Val-Gln-Arg (FCQR). It was demonstrated to be highly durable in tissue culture settings with a half-life expressed in days and to effectively suppress PAR2 activity (Swedberg et al., 2009).

6.1.3 KLK2 heptapeptide substrate (Acetyl-Gly-LysAla-Phe-Arg-Arg)

Acetyl-Gly-LysAla-Phe-Arg-Arg, a KLK2 heptapeptide substrate, has been conjugated with L12ADT to selectively target prostate cancer cells with thapsigargin (Janssen et al., 2006). Prodrug growth inhibition of KLK2 expressing LNCaP, CWR22R, and C4−2B cells was reported, and they have IC50 values ranging from 0.5 to 1 μM (Swedberg et al., 2010). This compound is relatively stable with a notable in vivo anticancer activity (Janssen et al., 2006)

6.1.4 L-377,202

L-377,202, N-glutaryl-(4-hydroxypropyl)-Ala-Ser-cyclohexaglycyl-Gln-Ser-Leu-CO_2H peptide, is covalently bound to the aminoglycoside part of doxorubicin and has great selectivity

for KLK3. The biologically active variants of L-377,202, leucine doxorubicin, are produced when PSA cleaves it at the Gln-Ser amide. In preclinical animal models, L-377,202 was found to be ~15 times more efficient than doxorubicin at stopping the growth of human prostate cancers, despite being less cytotoxic than the drug in KLK3-nonexpressing cells (DeFeo-Jones et al., 2000). Even when administered at four times the 100% lethal doxorubicin equivalent dose, L-377,202 showed no signs of systemic toxicity.

6.1.5 KLK3-activated TGX-D1

TGX-D1 is an analog of the extremely powerful phosphatidylinositol 3-kinaseβ (PI3Kβ) inhibitor. Zhu et al. (2008) and Signoretti et al. (2000) have demonstrated that the PI3K/Akt-mediated advancement of prostate cancers occurs through androgen independent status. However, the therapeutic applications of TGX-D1 are currently limited because of its poor solubility and the extensive distribution of PI3Kβ. A multicomponent peptide-based drug was developed by Signoretti et al. (2000). This drug has three different components: (1) TGX-D1, (2) a PSA cleavable peptide (SSKYQ), and (3) a HER2 (human EGF receptor 2)-binding domain. Since ~80% of CRPCs have overexpressed HER2, it seemed to be an useful tool to administer a medication to advanced prostate cancer cells using this marker (Signoretti et al., 2000). Upon entering the prostate cancer cells through HER-2, the inactive prodrug is quickly cleaved by PSA, releasing NH2-SL-TGX. This compound then proceeds to self-cycle, releasing the parent drug, TGXD1. Compared with the parent drug, this KLK3-activated prodrug showed much greater cellular absorption in LNCaP cells (Tai et al., 2011). These results indicated that this compound may have therapeutic value in CRPC.

6.1.6 KLK3-activated LY294002

The peptide Mu-LEHSSKLQL, whose internal sequence HSSKLQ serves as a selective substrate for PSA, was conjugated with LY294002, an analog of quercetin. In PSA-secreting C4-2 prostate cancer cells, this inhibitor dose and time-dependently decrease PI3K levels, which could result in cell death (Baiz et al., 2012). Before definitely demonstrating its efficacy as an efficient antitumor treatment, additional experiments in animal models of PCa are required to enhance the effective dispatch of the operative confirmation of LY294002 in the PCa cells.

6.1.7 BSD352

BSD352 has three functional domains: an anti-VEGF peptide (SP5.2), an antibasic fibroblast growth factor peptide (DG2), and a cell-penetrating domain taken from HIV transactivation of the regulatory protein (TAT) followed by the BH3 domain of the p53 (TAT-BH3). A PSA hydrolytic substrate peptide connected these various domains in BSD352 together. It was demonstrated that this complex construct could be effectively degraded by PSA in PSA generating LNCaP prostate cancer cells, leading to the upregulation of Bax, cytochrome C release, and caspase-9 cleavage, which ultimately promotes tumor cell apoptosis. It may be noted that a proven xenograft mice model could elicit up to 80% tumor growth inhibition when BSD352 was directly injected every 2 days for 14 days, along with a meaningful anti-angiogenic effect (Li et al., 2011). It has been shown that, in comparison with controls, BSD352 therapy resulted in a substantial decrease in CD31-positive blood vessels as well as a tumor cell death affecting 60% of the total tumor cell (Cereda et al., 2015).

6.1.8 PRX302

Sophiris Bio Corp. (California) developed PRX302, a recombinant agent of the native proaerolysin protein. Most mammalian cells surface normally binds native proaerolysin, which is then broken down into aerolysin by common membrane-bound proteases, for example, furins. Aerolysin oligomerizes quickly and permeates the cell membrane to create distinctly stable pores that causes rapid cell death (Abrami et al., 1998). A PSA-selective sequence has been inserted in place of the furin-cleavage sequence in proaerolysin through genetic modification (Williams et al., 2007).

Please refer to Table 16.1.

6.2 KLK-targeted miRNAs and siRNAs-/shRNAs

An intriguing novel strategy to target KLK activity involves the synthesis of synthetic single-strand DNA or RNA molecules known as aptamers. Aptamers work as antibodies against the KLKs (Mavridis et al., 2014). The way by which the prodrugs work is built up based on the release of various cytotoxic components in neoplastic tissues, where the activity of certain KLKs are visible (Table 16.1). Their use has two main benefits: low systemic toxicity and optimal tissue-specific delivery (Denny, 2004).

The RNA interference (RNAi) process is surely an useful technique to effectively reroute KLK expression. The two main components of RNAi are siRNA, or short hairpin RNA and miRNA. They have the ability to attach to mRNAs and reduce their activity by preventing mRNAs from generating the relevant protein. The therapeutic value of some endogenous miRNA in cancers has been demonstrated in a number of studies (Croce, 2009). Delivery of certain miRNAs has also been proven to slow the growth of prostate tumors in preclinical models (Mavridis et al., 2014). The benefits of miRNA in this regard are their small and, therefore, less antigenic size as well as their ability to target multiple genes. However, their influence on unrelated genes may cause systemic toxicities, which is their main drawback (Croce, 2009). Cancer therapy has made extensive use of exogenous delivery of siRNAs or shRNAs, which demand nearly perfect base-pairing with target sequences (Whitehead et al., 2009). In numerous preclinical models, KLK2, KLK3, and KLK4 knockdown in prostate cancer cells after siRNA or shRNA transfection has shown the best outcomes (Olkhov-Mitsel et al., 2012). Besides, many safety problems have been resolved by the coupling of siRNAs with liposomes, polymers, nanoparticles, or chemical modifications of oligonucleotides, which has recently replaced the outdated viral-based intracellular delivery strategy (Whitehead et al., 2009).

6.2.1 miR-143

The capacity of PC-3 cells to migrate and invade has been demonstrated to be inhibited by retrovirus transfection of miR-143 (and -145), which results in an increase in E-cadherin and a decrease in fibronectin on the surface of these cells. Additionally, it has been shown that PSA levels, bone metastasis, and Gleason score are inversely linked with downregulation of miR-143 (and miR-145) (Peng et al., 2011). It has been demonstrated that the rs4705342T > C functional polymorphism in the miR-143 promoter is associated with both a markedly elevated risk of prostate cancer and a suppression of miR-143 expression. On the other hand, restoring miR-143 levels may inhibit the expression of KLK2 mRNA and protein, as well as reduce the

TABLE 16.1 KLK-activated prodrugs have the following mechanisms of action: KLK-activated prodrugs can benefit prostate cancer cells through a variety of methods.

Prodrug	Constitution	Mode of action
KLK2-activated LD12ADT	LD12ADT is activated through substrate cleavage and upon entering, it increases intracellular calcium concentration by inhibiting sarco(endo)plasmic reticulum Ca^{2+} ATPase (SERCA).	Dysregulation of SERCA activity could produce cellular apoptosis.
L-377202	L-377202 is a highly selective KLK3 substrate that has been observed to be covalently attached to the aminoglycoside chain of doxorubicin (Doxo). The physiologically active forms of Doxo are of leucine doxorubicin (Leu-Doxo) and doxorubicin (Doxo) are generated upon cleavage of L-377202 by KLK3.	It generates free radicals, which causes damage to cellular membranes, DNA, and proteins. It also intercalates into DNA and impairs topoisomerase II-mediated DNA repair, thereby leading to cell death.
KLK3-activated TGX-D1	Three components of the peptide: TGXD1 (a strong PI3Kβ inhibitor), a HER2-binding domain, and a KLK3-specific substrate are observed to be associated with its pharmacological action. When an inactive prodrug reaches prostate cancer cells via HER-2, KLK3 cleaves the substrate and releases the parent drug, TGX-D1.	TGX-D1 suppresses the PI3K-AKT-mTOR pathway and thereby attenuates cell growth leading to cell death.
KLK3-activated LY294002	LY294002 conjugates a KLK3 substrate with a quercetin analog, which is then activated by KLK3.	It inhibits PI3K activity via competitive inhibition of an ATP-binding site on the p85α subunit.
BSD352	This compound has three functional domains: a cell-penetrating domain from HIV transactivating regulatory protein (TAT TD or transduction domain), BH3 domain of p53, an anti-VEGF peptide (SP5.2), and an antibasic fibroblast growth factor peptide (DG2). A KLK3 substrate peptide connects these distinct domains in BSD352.	This construct penetrates prostate cancer cells by TAT TD, is cleaved by KLK3, and triggers apoptosis via p53's BH3 domain. It suppresses neo-angiogenesis through SP5.2 while also blocking the tumor microenvironment via DG2.
PRX302	It consists of a KLK3 substrate and proaerolysin. It attaches to GPI-anchored proteins on mammalian cell surfaces and is cleaved by KLK3, resulting in the formation of aerolysin.	Aerolysin rapidly oligomerizes, generates stable pores, and causes cell death.

proliferation and migration of prostate cancer cell lines (Chu et al., 2016). These results point to a potential therapeutic use of mi-143 in the management of prostate cancer.

6.2.2 Anti-KLK4 siRNAs/shRNAs

It is well established that KLK4 plays a crucial role in triggering the PI3K/mTOR and AR pathways (Jin et al., 2013). Anti-KLK4siRNAs/shRNAs were shown to inhibit this signaling

pathway in prostate cancer cells and tissues (Darson et al., 1999). Initially, it was demonstrated that three distinct anti-KLK4 shRNAs significantly decrease cell growth in vitro when they were stably transfected into the three distinct prostate cancer cell lines: LNCaP, LAPC4, and VCaP. A marked decrease in tumor growth rate was observed in nude mice injected with ShKLK4-positive prostate cancer cells in comparison with the controls.

6.3 Vaccines and antibodies

6.3.1 PROSTVAC

The poxviral vaccine, PROSTVAC (Bavarian Nordic, Inc.), contains three costimulatory molecules in addition to PSA. These are leukocyte function-associated molecule-3 (LFA-3), intercellular adhesion molecule-1 (ICAM-1), and B-7.1 (B7-1 signals are essential for development of antigen-specific immune responses.) The PROSTVAC regimen consists of a recombinant fowl pox vector. Numerous investigations have indicated that this vaccination can provide a robust stimulation of helper T cells ($CD4^+$) and cytotoxic T cells ($CD8^+$), resulting in a notable reduction in the growth of prostate tumors (Madan et al., 2009).

7. Conclusion and future direction

This chapter indicates the role of KLKs, especially KLK2, KLK3, KLK4, and KLK14, in the development of prostate cancer and suggests that they could be considered as the promising targets for combination therapies for treatment of PCa. Further research needs to be focused on delving deeper into the functions of these proteases and the various ways in which they interact with other molecules. The malignant prostate secretes several KLKs, and understanding each KLK's temporal expression and activity as well as its proteolytic substrates and effector pathways appears to be crucial for the development of novel therapeutics of PCa. Furthermore, the majority of functional research has primarily examined the epithelial cell component; as a result, the interaction between KLKs and the nearby stroma has not yet received adequate attention.

Understanding how the tumor microenvironment regulates KLK activity will be essential to comprehending the function of KLKs at different stages of prostate cancer. Thus, to expedite their translation into the clinic, a deeper investigation of the KLK function in animal models is necessary. The main barriers to the clear success of engineered KLK inhibitors in human clinical trials are the high sequence similarities displayed by different KLKs (Mavridis et al., 2014; Réhault et al., 2001) and the documented broad range of KLK functions within the progression of prostate cancer (Coussens et al., 2002). Finding more potent medications and evaluating their toxicity and/or efficacy will be made possible by the optimization of substrate libraries and the creation of novel transgenic and knockout models.

A further promising therapy option was the use of compounds, which are able to stimulate specific effective immune responses against KLKs, based on the successful use of vaccines and immunotherapeutic agents in prostate cancer (Gerritsen, 2012). The promising future therapeutic approach for patients with prostate cancer includes anti-KLK3 antibodies conjugated with chemotherapeutic medicines, bispecific murine antibodies allowing both human

CD3 and KLK3, anti-KLK3 mouse/human chimeric IgE, and KLK-based immunotherapeutic vaccines (Mavridis et al., 2014).

KLK-targeted immunotherapeutic medications and KLK-activated prodrugs are currently the most promising treatments for PCa, each with a distinct set of advantages over other anti-KLK compounds. The application of substances enhancing the immune system's ability to fight cancer, such as vaccinations or antibodies, is designed to target KLKs. These drugs may be used to overcome resistance to conventional treatments. The significance of immune response downregulation during the advancement of prostate cancer has been recognized (Qian et al., 2009), and the recent success of sipuleucel-T in phase III clinical trials serves as justification for this (Kantoff et al., 2010). However, the primary disadvantages of sipuleucel-T therapy are its high cost, its production complexity, and the lack of a valid clinical metric to assess its effectiveness. Comparing PROSTVAC to sipuleucel-T treatment, the immunotherapy seems more viable. Although some clinical trials have demonstrated that the immune response to prostate cancer antigens, which were not originally targeted by the vaccine, may spread, PROSTVAC application has the drawback of being univalent against the targeted protein (PSA) (Gulley et al., 2014; Kantoff et al., 2010; Madan et al., 2009). The main obstacle to the application of immunotherapeutics in clinical settings, however, is the absence of reliable clinical response measures.

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CHAPTER

17

Mechanistic aspects of serine protease as biomarkers in wound healing of lung cancer

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1. Introduction

Proteases are the enzymes that are named according to their acting site, structure, and mechanistic pathways. It plays a very important role in biology as they are involved in different biochemical and physiological functions such as protein synthesis, adaptation, development and reproduction, and apoptosis. Protease also regulates motility and division. A number of proteases such as serine, cysteine, aspartic etc. are mainly present in the human lung. Among these proteases, serine proteases play a vital role to activate and protect the cells of the lung (Lecaille et al., 2016).

The death due to lung cancer is reported to be the maximum among the death due to cancer worldwide, with both identification and mortality rates rising sharply since the past 10 years. Despite ongoing advancements in diagnosis and treatment protocols, the patients with advanced non−small-cell lung cancer (NSCLC) have a low survival life because of metastases. Thus, identifying the molecular pathway was crucial to accelerate the course of NSCLC.

2. Classification

Serine proteases are classified in accordance with their site of action. Some examples of serine protease are as follows:

Chymotrypsin—It is one of the pancreatic digestive enzymes.

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Trypsin—This one is also a pancreatic digestive enzyme. It mediates different lung and liver injuries related to disease.
α-Chymotrypsin—This enzyme mediates Parkinson's and Alzheimer's disease.
Elastase—It is also a pancreatic digestive enzyme.
Urokinase—This serine protease is used to mediate a number of conditions such as critical conditions of glomerular, kidney, cardiovascular and critical obstructive pulmonary disease, epilepsy, breast and prostate cancer.
Plasmin—This type of serine protease enzyme dissolves blood clots and mediates in encephalomyelitis, conjunctivitis, coronary artery disease, and colitis.
Thrombin—This type of serine protease enzyme activates fibrinogen to form blood clots. It also accelerates in mediating the following critical conditions: cardiovascular, chronic obstructive pulmonary and Alzheimer's disease, sickle cell disease and pneumococcal meningitis, etc. (Patel, 2017).

3. Mechanism of action of serine protease

Many different biological activities utilize serine proteases. The mechanistic pathways of interacting protease inhibitors have been extensively studied by various investigators

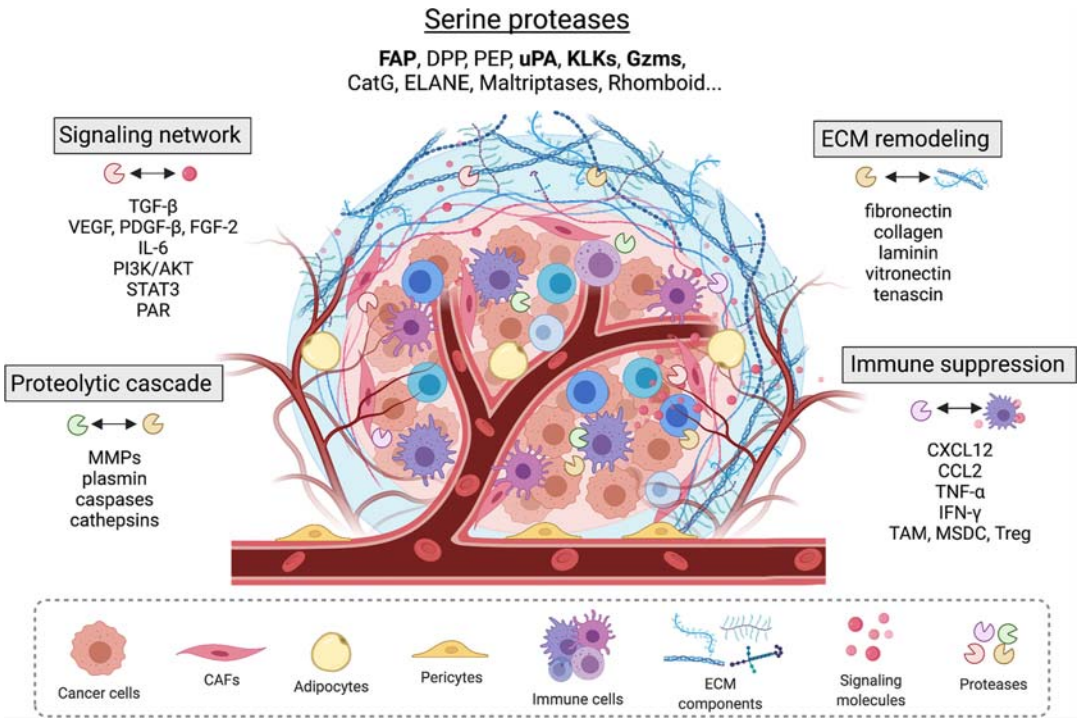

FIGURE 17.1 Mechanism of action of serine proteases found in the tumor microenvironment. *Reprinted from Tagirasa and Yoo (2022).*

(Tagirasa and Yoo, 2022). Several protease inhibitors can engage with proteases in tumor microenvironments (Fig. 17.1).

Mode of action of serine proteases in a tumor environment is shown in Fig. 17.1.

The inhibitors are generally unable to recover their original structure since the reactions are irreversible. As a result, suicide inhibitors are frequently used to refer to inhibitors implicated in trapping reactions (Eguchi et al., 1993). The other often seen mode of protease−inhibitor interaction is the tight-binding response, or standard procedure, which was extensively detailed by Laskowski and Qasim (2000) and subsequently by Farady and Craik (2010). The canonical inhibitors are those that work via this mechanism and are primarily being described for serine protease inhibitors. The inhibitors from plant sources primarily adhere to the conventional inhibitory mechanism.

The capacity of tumor cells to penetrate healthy tissues is one of their defining characteristics (Singh and Gray, 2021). The destruction of cell−cell and cell−matrix bonding, as well as the breakdown of ECM elements, are just a few of the mechanisms that contribute to the invasion and migration processes. Emphysema development occurs due to lack of the inhibitor, α1-PI.

The tumor microenvironment relies on the extracellular matrix (ECM) for more than just acting as a physical wall to tumor aggression (Yang et al., 2008). The malignant cells are heightened in proteolytic activity, and it makes them able to break down the extracellular matrix (Kim et al., 2011). By binding to specific molecules within the intercellular substance, these cells can trigger neighboring cells to produce various degrading enzymes, facilitating the malignant cells penetration into the bloodstream through the extracellular matrix (Aumailley and Gayraud, 1998). For cancer cells to penetrate and move through the basal lamina, which is a major feature of malignancy, this digestive process is essential. Cancer cells' aggression and migration can eventually result in the development of metastases in distant areas. In contrast, proteases play an important function in a variety of biological processes in normal cells, including gene expression, differentiation, and cell death (Chakraborti et al., 2017). Since they can destroy the proteins and extracellular matrices, proteases are also closely linked to the development of cancer in both primary and metastatic sites. As both positive and negative factors of vascular development and endothelial expansion of cells, ECM-degrading proteases are also essential for angiogenesis. Additionally, tumor-specific protease activation can be used to reduce immunotherapeutic drug toxicity and side effects by limiting systemic exposure (Hussain et al., 2020).

Proteolytic enzymes called serine proteases control a variety of normal and pathological functions. Several mammalian tissues, including the skin, prostate gland, and semen, have been reported to have a membrane-anchored serine protease (PRSS8). Ma et al. (2017) have examined the expression, biological role, and associated mechanism of action of protease serine S1 family member 8 (PRSS8) in the pathophysiology of NSCLC. The findings showed that PRSS8 was only slightly expressed in NSCLC cell lines and that, as shown by the ectopic expression, it prevented the formation of tumors in human NSCLC both in vitro and in vivo. From the measurement of the expression level of PRSS8 in NSCLC cell lines, such as A549, PC9, and NCI−H1993, it was revealed that the m-RNA levels in human NSCLC cell lines were significantly low compared with those in a normal human bronchial epithelial cell line (BEAS-2B). Results from the Matrigel invasion experiment and Transwell Migration assay clearly show that ectopic PRSS8 expression prevented A549 cells from invading Fig. 17.2. Western blot analysis was used to explore the molecular mechanism of PRSS8's suppression of NSCLC cell proliferation and metastasis in A549

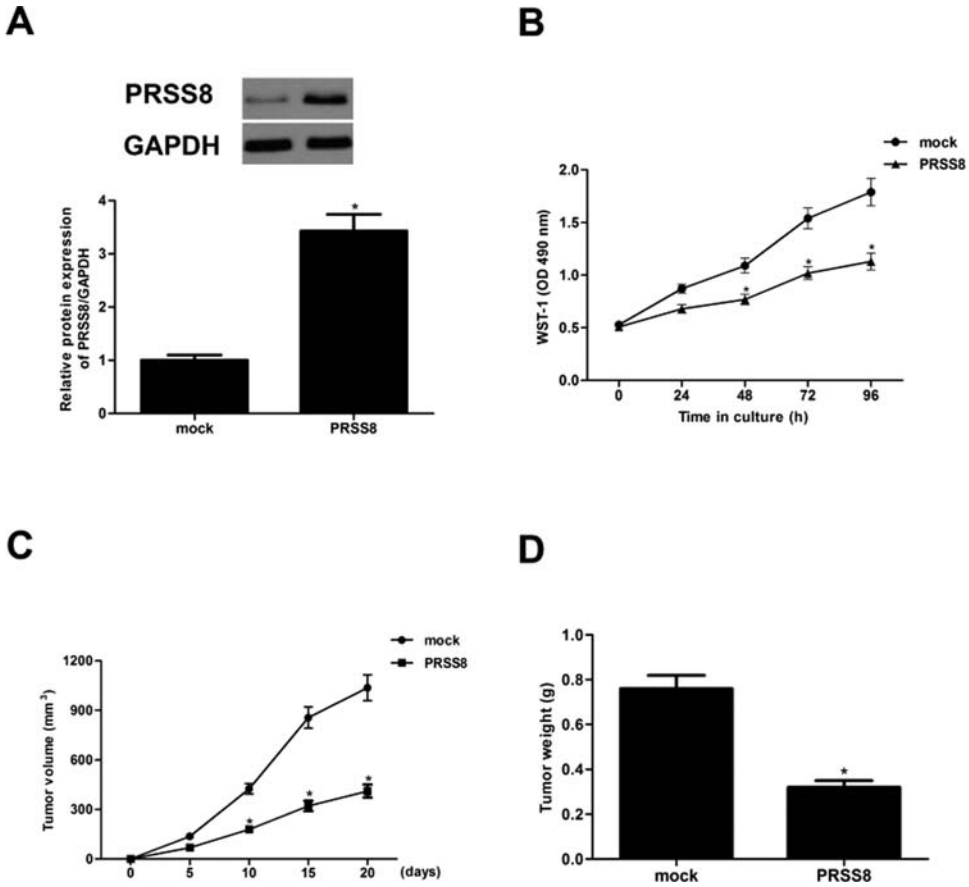

FIGURE 17.2 Ectopic expression of PRSS8 reduced tumor development both in vitro and in vivo. A549 cells were transfected with either PRSS8 or a mimic for 24 h. (A) Western blotting and qRT-PCR analysis to determine the corresponding transfection efficiency. (B) The WST-1 assay to measure the proliferation of cells. Four-week-old female BALB/c-nu mice were given subcutaneous injections of PRSS8 cells (5×10^6) and control cells into their left and right dorsal flanks. (C) Tumor volume was recorded every 5 days. (D) The tumors were removed and weighed when the animals were slaughtered after 20 days. Triplicate runs of the experiments were conducted. $^*P < .05$ in comparison with the mock group. *Reprinted from Ma et al. (2017).*

cells, as well as the influence of PRSS8 on JAK/STAT 3 activation to check the impact of PRSS8 on tumor development both in vitro and in vivo (Fig. 17.3).

In vitro and in vivo ectopic expression of PRSS8 minimized tumor development (A) Western blotting and qRT-PCR analysis to determine the corresponding transfection efficiency (B) The WST-1 assay to measure the proliferation of cells is shown in Fig. 17.2.

The expression of PRSS8 in minimum level of NSCLC cell lines (A) qRT-PCR analysis to assess the PRSS8−mRNA expression in human NSCLC cell lines (B) Western blot analysis is shown in Fig. 17.3.

Following PRSS8 transfection, A549 cells showed elevated PRSS8 protein and mRNA levels. When compared with the mock group, the WST-1 assay results showed that ectopic

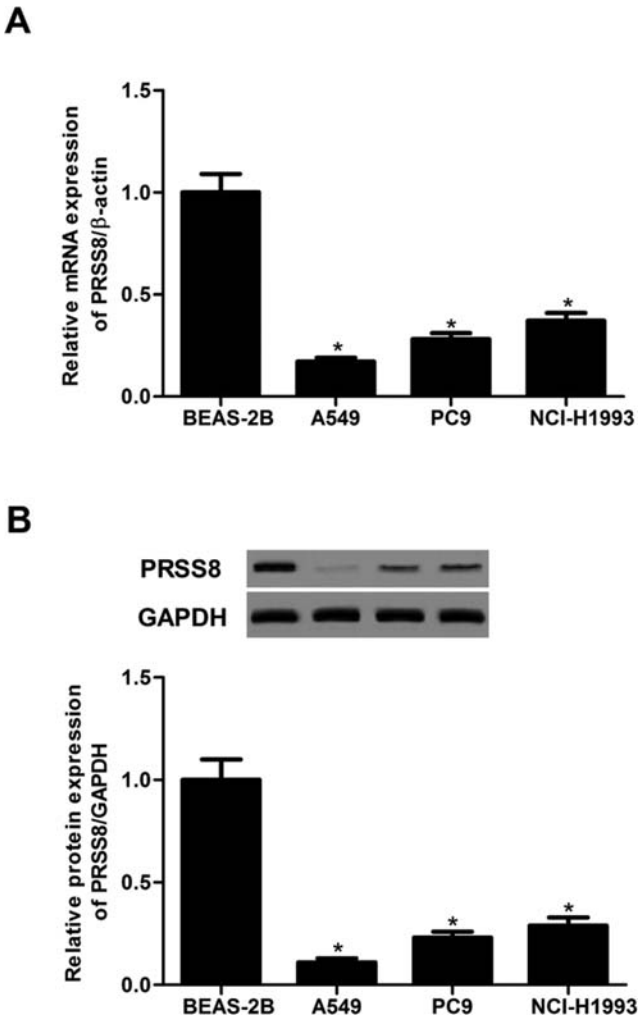

FIGURE 17.3 PRSS8 expression in low level of NSCLC cell lines. (A) qRT-PCR analysis to assess the PRSS8−mRNA expression in human NSCLC cell lines. (B) Western blot analysis to examine the protein expression of PRSS8 in human NSCLC cell lines. Triplicate runs of the experiments were conducted. $^{*}P < .05$ in comparison with BEAS-2B. *Reprinted from Ma et al. (2017).*

expression suppressed the proliferation of A549 cells. A xenograft implanting human NSCLC in nude mice was used to examine the function of PRSS8 in NSCLC in vivo. From the result, it was seen that the PRSS8-treated group had a mean subcutaneous tumor that was smaller than the mock group.

In a separate study, lung cancer cells were reported to internalize serine proteases neutrophil elastase (NE) and proteinase 3 (P3) from various sources, although uptaking of P3 and NE by lung cancer cell lines has been reported earlier (Gregory et al., 2012; Houghton et al., 2010). First, as shown in Fig. 17.4, it was verified that a panel of lung cancer cell lines did not naturally express the complete transcripts of NE and P3 (Barretina et al., 2012). However, upon coincubation with external sources, lung tumor cells may contain NE and P3 protein; the H2023 cell line absorbed NE and P3 from soluble, pure proteins and healthy

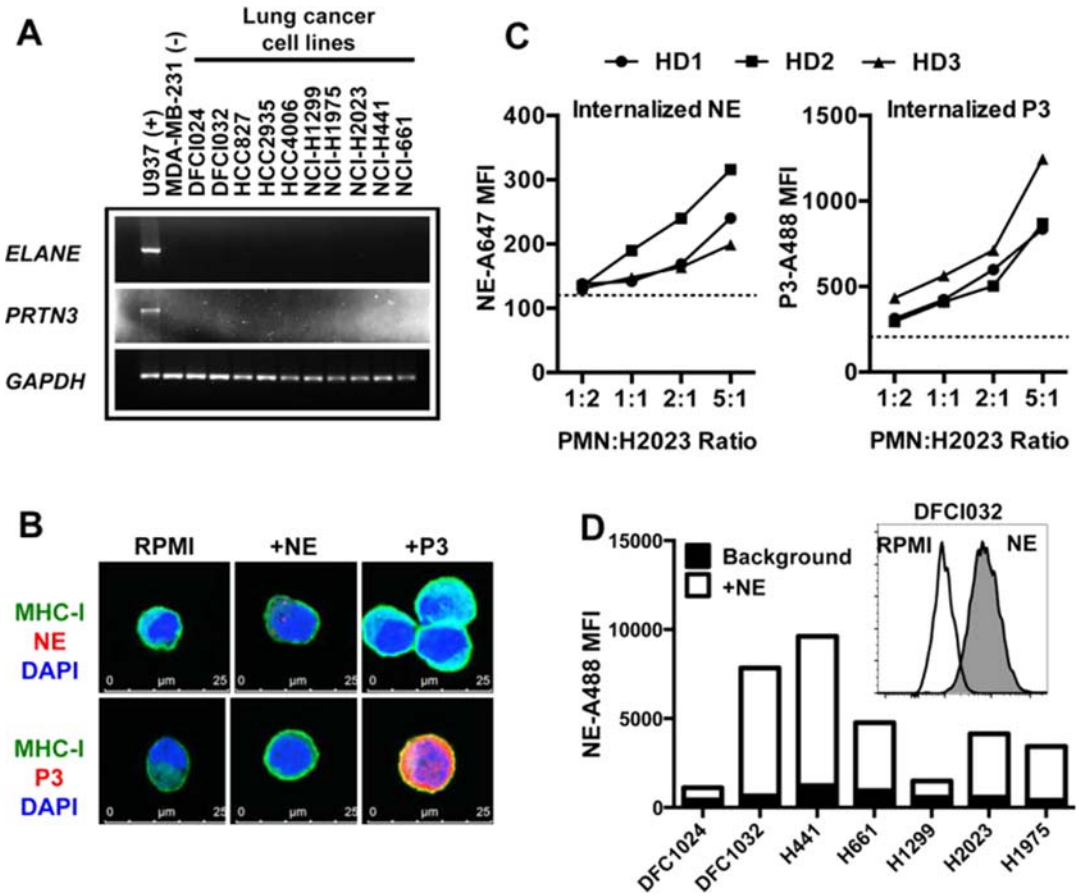

FIGURE 17.4 Exogenous serine proteases are internalized by lung cancer cells. (A) Determination of RT-PCR test showed that lung cancer cells do not express full-length P3 (PRTN3) or NE (ELANE) transcripts. (B) Confocal imaging of internalized proteins in H2023 lung cancer cells that possess the NE, P3, and PR1 epitope. (C) Flow cytometry diagram of internalized NE and P3 from three different peripheral mononuclear (PMN) lysates by H2023 from healthy donors (HD); the dotted line shows unpulsed cell labeling. (D) Flow cytometry diagram determining the uptake of pure NE in a panel of lung cancer cells with autofluorescence removed; the inset displays DFCI032 as an example histogram with NE (*gray*) and without (*white*, RPMI). *Reprinted from Peters et al. (2017).*

donor-PMN. We expanded on these results using pure NE in a panel of lung cancer cell lines. These findings show that whereas lung cancer cells can absorb NE and P3 from external sources, they cannot produce these molecules endogenously.

Illustration of exogenous serine proteases being internalized by lung cancer cells is shown in Fig. 17.4: (A) Determination of RT-PCR test. (B) Confocal imaging of internalized proteins in H2023 lung cancer cells. (C) Flow cytometry diagram. (D) Flow cytometry diagram.

Serine proteases improve antigen presentation by upregulating surface expression of the human leukocyte antigen (HLA class I). It has been demonstrated that breast cancer and other solid tumors cross-present the immunogenic, HLA-A2-restricted leukemia-associated

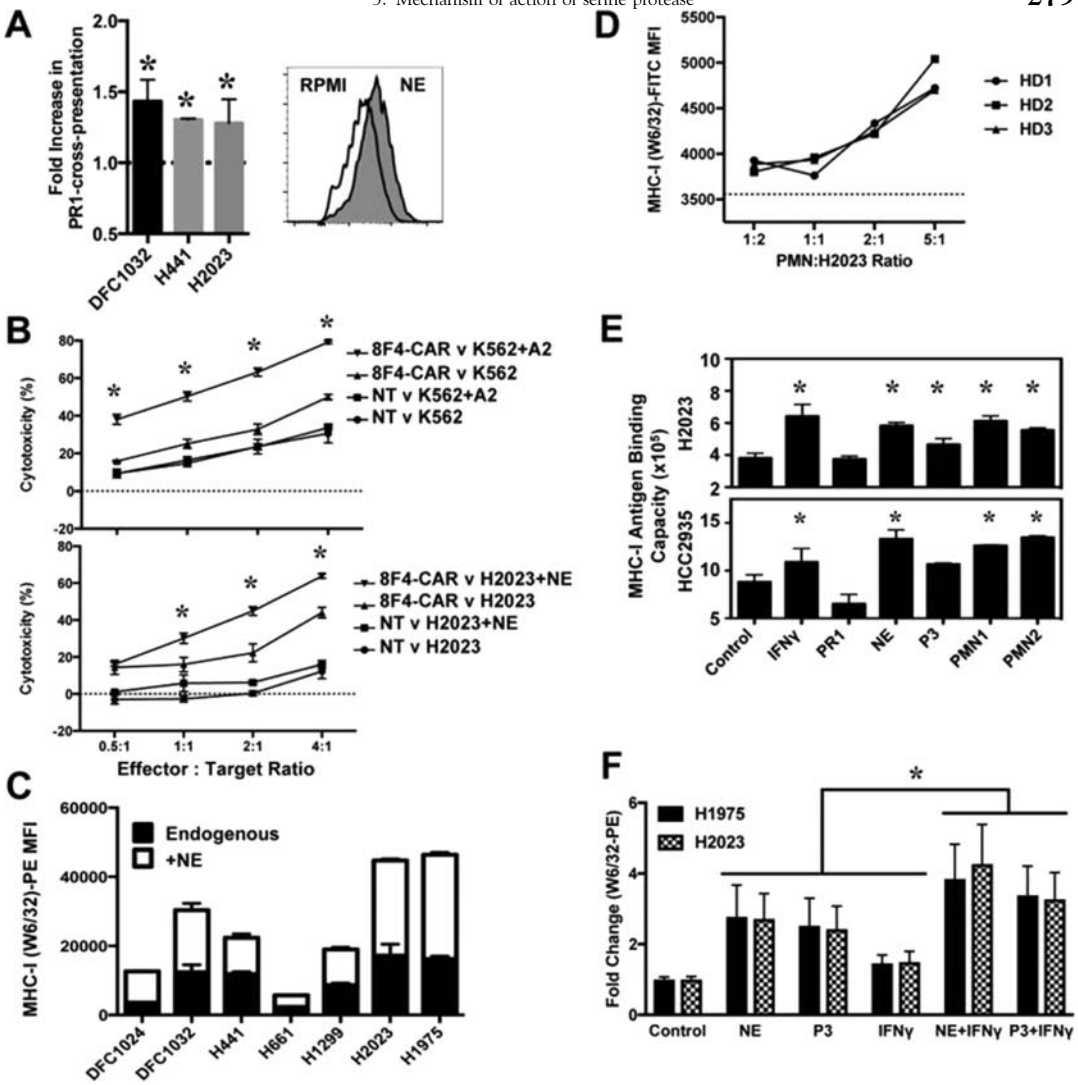

FIGURE 17.5 P3 and NE improve the presentation of antigens. (A) After coincubating with NE for 24 h, the immunogenic PR1 peptide on HLA-A2+ lung cancer cells increased. This was measured using flow cytometry, which is based on the binding of the 8F4 antibody (anti-PR1/HLA-A2) directly conjugated to AlexaFluor647. The increase is shown as a fold-increase in PR1 cross-presentation over baseline. An example DFCI032 histogram with NE (*gray*) and without (*white*, RPMI) is shown in the inset. (B) H2023 lung cancer cells exposed to NE are lysed by CAR-T cells that recognize PR1/HLA-A2 from healthy donors. As effector controls, nontransduced T cells (NT) and K562 cell lines that expressed NE and P3 endogenously and were transduced with HLA-A2 to enable PR1 presentation were employed. The graph shows the average of two independent experiments' three technical replicates; asterisks denote significant differences between the target cell populations of 8F4-CAR-transduced effectors by the two-way ANOVA with $P = .05$ as the criterion for significance. (C) Using W6/32, an antibody to folded HLA-A, B, and C, flow cytometry was used to detect the increased total HLA class I surface expression in purified NE and (D) NE provided by PMNs from healthy donors. (E) HLA class I increased surface is influenced by NE. The following media were used to incubate lung cancer cells for 24 h: Lysates from healthy donor PMN at a 2:1 PMN:target cell ratio, pure NE or P3 (10 μg/mL), PR1 peptide (10 μg/mL, negative control), and IFNγ (100 U/mL, positive control). Asterisks indicate a substantial increase over the initial HLA class I antigen binding capacity determined by two-way ANOVA, using a significance threshold of $P = .05$. (F) Surface MHC-I is upregulated by the synergy of serine proteases NE and P3 and IFNγ. Cells processed as experiments performed in triplicate are shown. Asterisk indicates a significant increase in surface MHC-I of combined treatments compared with respective single treatments alone by the two-way ANOVA with $P = .05$ as the criterion for significance. *Reprinted from Peters et al. (2017).*

antigen PR1, which is found in the serine proteases NE and P3. No reports of lung cancer cells expressing the exogenous antigen PR1 exist, despite the fact that lung cancer has been demonstrated to internalize NE (Houghton et al., 2010). It was observed that the interaction of PR1 from NE by HLA-A2+ lung cancer cells via flow cytometry, as demonstrated earlier in antigen-presenting cells, breast cancer, and melanoma (Alatrash et al., 2012a,b), using the PR1/HLA-A2-specific monoclonal antibody 8F4 (Sergeeva et al., 2011, 2016). Furthermore, lung cancer cells exposed to NE were more vulnerable to being destroyed by cytotoxic T lymphocytes (CTL) that carried the 8F4-chimeric antigen receptor (CAR) that is specific to PR1. Lung cancer cells were therefore able to cross-present PR1 when exogenous NE was present and were vulnerable to immunotherapies that targeted PR1 (Fig. 17.5).

The presentation of antigens being improved by P3 and NE is shown in Fig. 17.5: (A) After coincubating with NE for 24 h, the immunogenic PR1 peptide on HLA-A2+ lung cancer cells increased. (B) H2023 lung cancer cells exposed to NE are lysed by CAR-T cells that recognize PR1/HLA-A2 from healthy donors. (C) Using W6/32, an antibody to folded HLA-A, B, and C, flow cytometry was used to detect the increased total HLA class I surface expression in purified NE. (D) NE provided by PMNs from healthy donors. (E) HLA class I increased surface is influenced by NE. (F) Surface MHC-I is upregulated by the synergy of serine proteases NE and P3 and IFNγ.

After mixing with NE, it was shown a boost in surface HLA class I molecules on lung cancer cell lines and breast cancer cell lines (Chawla et al., 2016). The results were confirmed by detecting HLA class I molecule expression on all lung cancer cell lines analyzed, which rose following exposure to NE, using an antibody specific for folded, pan-HLA-A,B,C (mAb W6/32). Within the microenvironment of lung cancer tumors, NE is encapsulated in azurophilic granules and transported to the tumor by means of TAN and TAM, which, upon activation, release the serine proteases enclosed in the granules. As the ratios of PMN to tumor cells increased, there was a dose-dependent increase in surface HLA class I expression, which supported the elevation in surface HLA class I expression. Moreover, the study shows that NE enhances HLA class I surface expression in lung cancer more than P3 does, and it works in concert with the proinflammatory cytokine IFNγ to raise MHC class I on the outermost layer of lung cancer cells.

4. Conclusion

From the discussion, it is clear that the PRSS8 played a vital part in the development and metastasis of NSCLC and prevented the tumor development in vitro and in vivo in human NSCLC cells as evident through the ectopic expression data and JAK/STAT3 signaling pathway. We have seen that the expression levels of PRSS8 in both mRNA and protein were markedly downregulated in human NSCLC cell lines. In summary, it was shown that the lung cancer cells were more receptive to breakage by CTL that targeted these peptide/HLA-A2 and that PMN/macrophage-associated NE increased antigen presentation by lung cancer cells, particularly the foreign antigen PR1 and produced endogenous antigens. Furthermore, it was demonstrated that the presence of CTL specific to NE-induced endogenous antigens in the lung cancer patients, especially in the TIL populations.

Immunocheckpoint blockage and PR1-based immunotherapies, including CAR-T cells, monoclonal antibodies, and vaccinations, may thus be very beneficial for lung cancer patients, especially those with high levels of TAN and TAM. Lastly, immunotherapies based on PR1 may work in concert with immunocheckpoint inhibition to provide lung cancer patients significant, long-term advantages.

In the coming years, a significant area of investigation will focus on identifying the physiological targets of hemolymph protease inhibitors. During the progression of malignancy, several serine proteases emerge as important players at the interface between the tumor and its surrounding stroma. These proteases function in multiple ways, not only interacting with other proteases but also influencing crucial signaling pathways involved in the activation or inactivation of chemokines, cytokines, kinases, and growth factors. The role of serine proteases as emphasized in this chapter provides typical discoveries from the recent past. To uncover more appropriate diagnostic indicators and therapeutic targets, it is imperative to continue studying the complex roles played by proteases within the tumor microenvironment, including their impact on extracellular matrix degradation. Specifically, in the context of antitumor immunity, whether it occurs naturally or is induced by immunotherapy, understanding the involvement of these serine proteases in modulating immune responses on stromal cells will contribute to advancing pharmaceutical strategies.

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CHAPTER

18

The role of acetylcholinesterase in cancer development and possible therapeutic applications

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1. Introduction

The cholinergic system is primarily responsible for regulating learning and memory processes in the brain. This system consists of several key components, including the neurotransmitter acetylcholine (ACh), enzymes such as acetylcholinesterase (AChE), butyrylcholinesterase (BuChE), and choline acetyltransferase (ChAT). It also involves specific receptors known as muscarinic acetylcholine receptors (mAChR) and nicotinic acetylcholine receptors (nAChR), along with the vesicular acetylcholine transporter (VAChT). Together, these components form the intricate network that facilitates essential cognitive functions in the brain (Deiana et al., 2010).

The cholinergic system has been found to play a significant role in cancer development and progression. It is known to influence various aspects of tumor biology, including cell proliferation, angiogenesis, migration, invasion, and survival, all of which are essential processes for cancer growth (Paleari et al., 2008).

In cancer cells, the cholinergic system can be dysregulated, leading to altered levels of ACh. Changes in the expression and activity of enzymes involved in ACh synthesis, such as ChAT, and enzymes responsible for ACh degradation, such as AChE, have been reported in different cancer types. These alterations can result in imbalances in ACh levels and disrupted cholinergic signaling, contributing to tumor progression (Castillo-González et al., 2015).

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Cholinergic signaling in cancer cells often involves binding ACh to mAChRs and nAChRs on the cell surface. Activation of these receptors can trigger various intracellular signaling pathways that promote cancer cell survival, proliferation, and metastasis (Castillo-González et al., 2015).

Disruptions in the cholinergic system have been implicated in various diseases, including Alzheimer's disease (Mitić and Lazarević-Pašti, 2021), depression (Tasić and Lazarević-Pašti, 2022), Parkinson's disease (Bohnen and Albin, 2011), schizophrenia (Terry, 2008), and neuromuscular disorders (Ofek and Soreq, 2012). As a result, drugs that modulate cholinergic signaling, such as AChE inhibitors, are used in the treatment of neurodiseases and cancer. Additionally, research continues to explore the potential therapeutic applications of targeting the cholinergic system in various other disorders and medical conditions (Lazarević-Pašti et al., 2017).

This chapter discusses the pivotal role of cholinergic system components in cancer development, with particular emphasis on exploring AChE activity modulators as potential therapeutic interventions in cancer treatment.

2. AChE structure and function

AChE is an enzyme that plays a critical role in breaking down the neurotransmitter ACh within the synaptic cleft. It is an essential component of the cholinergic system, responsible for transmitting nerve signals. The structure of AChE consists of a globular protein composed of a monomeric subunit. It primarily comprises a catalytic core surrounded by peripheral anionic and hydrophobic regions, resulting in a twisted, ellipsoid shape. The catalytic core of AChE contains the active site where the enzymatic hydrolysis of ACh occurs. It forms a narrow and deep crevice-like structure known as a gorge. Specific amino acid residues within the active site facilitate the catalytic reaction (Colović et al., 2013). The catalytic mechanism of AChE involves a serine residue (Serine 200 in human AChE) initiating a nucleophilic attack on the ACh molecule. It leads to the cleavage of the acetyl group, resulting in the formation of choline. This mechanism is commonly referred to as the "serine hydrolase" mechanism. The peripheral regions of AChE play essential roles in regulating and stabilizing the enzyme (Bondžić et al., 2020; Patocka et al. 2004). The anionic peripheral region interacts with the cell membrane, anchoring AChE to the cell surface, and the hydrophobic peripheral region contributes to stabilizing the protein structure and facilitating protein–protein interactions (Johnson and Moore, 2006). AChE exists in various molecular forms, including monomers, dimers, and tetramers. These different forms exhibit distinct properties and can be found in different tissues and cell types (Massoulie and Bon, 1982).

2.1 AChE isoforms

The human AChE gene spans approximately seven kilobases and is located at position q22 on chromosome 7 (7q22). It consists of six exons and five intronic regions. Alternative splicing of the AChE gene leads to the production of different AChE isoforms with varying C-terminal regions: AChE-S (synaptic), AChE-H (hydrophobic or erythrocyte), and AChE-R (read-through). These isoforms have been identified in human cancers and are

characterized by alternative exons 4–6 (E4–E6) forming different C-terminal motifs. Distinct isoforms of AChE are generated through alternative splicing of the AChE gene. This chapter focuses on describing three major AChE isoforms: AChE-S (synaptic), AChE-H (hydrophobic or erythrocyte), and AChE-R (read-through). These isoforms have been found in human cancers and are characterized by different C-terminal motifs formed by alternative exons 4–6 (E4–E6) (Sternfeld et al., 1997).

AChE-S, also known as AChE-T, is a tetrameric protein formed by the fusion of exon 4 with exon 6. The C-terminus of AChE-S contains cysteine residues that form disulfide bridges, contributing to the tetrameric structure. AChE-S interacts with collagen Q (ColQ) and anchors itself to the neuromuscular junction. AChE-S binds to a proline-rich membrane anchor protein (PRiMA) in the brain, facilitating its association with cell membranes. An extended variant of AChE-S called N-AChE-S has been identified in the brain. N-AChE-S has its core domain outside the cell, while its extended N-terminus is in the cytoplasm (Feng et al., 1999).

AChE-H, also known as AChE-E, is produced by directly splicing exon 4 to exon 5. AChE-H is a dimeric protein that contains a glycophosphatidylinositol (GPI) group, anchoring it to the membrane of erythrocytes. Further alternative splicing generates the monomeric AChE-R protein. AChE-R is a soluble protein distributed extensively in the synaptic cleft. The expression patterns of AChE isoforms vary in normal and diseased states, suggesting their potential as molecular targets in neuromuscular and neurodegenerative diseases (Grisaru et al., 2001).

The expression patterns of AChE isoforms vary in normal and diseased states, indicating that they are involved in cellular stress and apoptosis and may serve as targets for therapeutic interventions in neuromuscular and neurodegenerative disorders. When a nerve impulse reaches the presynaptic terminal of a cholinergic neuron, it triggers the release of ACh into the synaptic cleft. ACh then binds to specific receptors on the postsynaptic membrane, facilitating the transmission of the nerve signal. However, to ensure proper signal transmission and termination, regulating the concentration of ACh is crucial (Grisaru et al., 2001).

It is where AChE plays a significant role. By breaking down ACh, AChE prevents its continuous accumulation within the synaptic cleft, enabling precise control of cholinergic signaling.

The cholinergic function of AChE encompasses several crucial roles. It rapidly degrades ACh, terminating its action and ensuring the availability of postsynaptic receptors for subsequent nerve impulses. AChE also controls the levels of ACh, regulating the strength and duration of cholinergic signals to maintain proper neurotransmission and prevent excessive stimulation. Additionally, AChE prevents the accumulation of ACh in the synaptic cleft, promoting precise communication between neurons and minimizing interference with neighboring synapses. By modulating cholinergic signaling, AChE fine-tunes the overall cholinergic tone, influencing the duration and intensity of cholinergic neurotransmission. In summary, AChE's cholinergic function ensures precise and controlled neurotransmission by regulating ACh levels, terminating signals, regulating strength, preventing interference, and fine-tuning cholinergic activity (Giacobini, 2003; Lu et al., 2012).

AChE, primarily recognized for its involvement in cholinergic neurotransmission, has recently been found to have noncholinergic functions in diverse tissues and cell types, contributing to various physiological processes. Some examples include its participation in cell adhesion and migration, where it interacts with adhesion molecules, facilitating

interactions between cells and the extracellular matrix. AChE also plays a role in neuronal development and plasticity by interacting with growth factors and contributing to synapse formation. Additionally, it exhibits neuroprotective and antiinflammatory effects, reducing oxidative stress and modulating inflammatory responses. It is involved in blood coagulation through its interaction with platelets, influences cellular signaling pathways, and can regulate immune responses in the central nervous system and peripheral tissues. These findings highlight the multifaceted nature of AChE beyond its cholinergic function (Balasubramanian and Bhanumathy, 1993; Lu et al., 2012).

The ACh pathway encompasses a series of events involved in the synthesis, release, receptor binding, and subsequent signaling of ACh in the nervous system. This pathway can be categorized into several key steps. ACh is synthesized within nerve terminals through the enzymatic action of choline acetyltransferase (ChAT). ChAT facilitates the transfer of an acetyl group from acetyl-CoA to choline, producing ACh. Once synthesized, ACh is packaged into vesicles within the nerve terminal, awaiting release. These vesicles, containing ACh, are transported to the presynaptic membrane, ready for release upon neuronal stimulation. The arrival of an action potential at the presynaptic terminal depolarizes the membrane and triggers the opening of voltage-gated calcium channels. Calcium influx into the presynaptic terminal leads to the fusion of ACh-containing vesicles with the presynaptic membrane, enabling the release of ACh into the synaptic cleft through exocytosis. Released ACh diffuses across the synaptic cleft and binds to specific receptors situated on the postsynaptic membrane. Two primary types of ACh receptors are muscarinic receptors (coupled to G-proteins) and nicotinic receptors (ligand-gated ion channels). The specific receptor subtype activated depends on the synapse's location and function. ACh binding to muscarinic receptors triggers intracellular signaling cascades mediated by G proteins, leading to various cellular responses based on the receptor subtype and tissue involved. Activation of nicotinic receptors results in the opening of ion channels, allowing the influx of cations (such as sodium) and depolarization of the postsynaptic membrane. ACh signaling is terminated through various mechanisms. One crucial mechanism involves the enzymatic breakdown of ACh by AChE present in the synaptic cleft. AChE hydrolyzes ACh into choline and acetate, preventing its prolonged action on receptors. Choline is subsequently taken back into the presynaptic terminal via a high-affinity choline transporter to be reused in ACh synthesis (Amenta and Tayebati, 2008; Takahashi, 2021).

The ACh pathway plays a vital role in the normal functioning of the nervous system, influencing processes such as muscle contraction, cognitive function, autonomic regulation, and sensory processing (Amenta and Tayebati, 2008). As many environmental pollutants are shown to inhibit AChE, exposure to them can lead to disruption or dysregulation of the ACh pathway and therefore contribute to various neurological and neuromuscular disorders (Anićijević et al., 2023; Lazarević-Pašti et al., 2023; Milanković et al., 2023; Tasić et al., 2023).

The ACh pathway has been implicated in cancer development and progression.

The ACh pathway involves the release of ACh from cholinergic neurons and its binding to muscarinic and nicotinic receptors on target cells. Cholinergic signaling is known to play a role in cell proliferation, angiogenesis, migration, invasion, and survival, all of which are relevant to cancer development and progression. Dysregulation of the ACh pathway can occur in cancer. Various cancer types have reported changes in the expression and activity of ACh-synthesizing enzymes (ChAT) and ACh-degrading enzymes (AChE). These alterations can

lead to imbalances in ACh levels and disrupted cholinergic signaling. Muscarinic receptors are widely expressed in different tissues and have been implicated in cancer. Activation of muscarinic receptors can stimulate various signaling pathways, which can promote cancer cell proliferation, survival, migration, and angiogenesis (Calaf et al., 2022). Nicotinic receptors are predominantly found in the nervous system but can also be expressed in nonneuronal tissues, including cancer cells. Activation of nicotinic receptors can lead to calcium influx and the activation of downstream signaling pathways that influence cell growth, proliferation, and migration (Egleton et al., 2008). The ACh pathway can also impact the tumor microenvironment, including immune cell functions. Cholinergic signaling has been shown to regulate immune cell responses, such as T cell activation, differentiation, and cytokine production. Dysregulation of cholinergic signaling in the tumor microenvironment can affect antitumor immune responses and contribute to immune evasion (Tan et al., 2021).

Targeting the ACh pathway components has been explored as a potential therapeutic strategy in cancer treatment. Inhibitors of ACh synthesis or antagonists of muscarinic and nicotinic receptors have been investigated in preclinical and clinical studies to modulate cholinergic signaling and potentially inhibit tumor growth and progression. It is important to note that the specific role of the ACh pathway in cancer can vary depending on the cancer type, stage, and microenvironment. The interplay between cholinergic signaling and other signaling pathways within the tumor microenvironment is complex and context-dependent (Lazarević-Pašti et al., 2017).

The variation in AChE activity across different cancer types likely stems from the specific functioning of the cholinergic network in each cell type. Additionally, differences in AChE activity among patients with the same type of cancer may be attributed to individual variability.

3. AChE in apoptosis

The cholinergic system is directly involved in apoptosis, a process that eliminates cells to maintain balance in development, differentiation, and aging. Dysregulation of apoptosis leads to diseases, particularly cancer. There are two controlled pathways: the extrinsic pathway through death receptors and the intrinsic pathway through mitochondria. Ligand activation of death receptors initiates caspase-8 activation and apoptosis in the extrinsic pathway, while the intrinsic pathway responds to intracellular death signals and activates caspase-9. Both pathways converge to activate executioner caspases, triggering irreversible apoptosis. However, not all cells undergo cell death and abnormalities in cell death regulation can result in various disorders, primarily cancer (Zhang and Greenberg, 2012; Zhang et al., 2002).

AChE, primarily known for its role in the termination of cholinergic signaling, has also been implicated in apoptosis. AChE has been shown to participate in apoptotic signaling pathways as it interacts with various proteins involved in apoptosis, such as Apaf-1 (apoptotic protease-activating factor 1) and cytochrome *c*, to promote the formation of the apoptosome, a multiprotein complex responsible for activating caspases and initiating apoptosis. AChE's involvement in apoptosome formation and activation of downstream caspases highlights its role in facilitating apoptotic cell death (Ye et al., 2010). It has been

suggested that AChE possesses tumor-suppressive properties through its involvement in apoptosis. In various cancer types, including colon carcinoma, hepatocellular carcinoma, and lung cancer, AChE expression is reduced compared with normal tissues. Decreased AChE levels may compromise apoptotic signaling and contribute to tumor growth and progression. It also can influence cell survival by regulating ACh levels. AChE hydrolyzes ACh, thereby decreasing its availability. In certain conditions, such as exposure to stress stimuli or chemotherapeutic drugs, the expression of AChE can be upregulated. Elevated AChE levels can modulate ACh signaling and promote apoptosis, leading to cell death. Conversely, decreased AChE activity may contribute to enhanced cell survival and resistance to apoptotic signals (Park et al., 2004; Xi et al., 2015).

Unlike AChE-S, which promotes cell death, AChE-R has the opposite effect and acts as a positive regulator of cell proliferation. Generally, AChE has a proapoptotic function. It is upregulated in response to various apoptotic stimuli, thereby promoting apoptosis. Conversely, reducing AChE expression through methods such as antisense RNA (asRNA), small interfering ribonucleic acids (siRNAs), or heterozygous deletion of the AChE gene attenuates apoptosis. These findings suggest that the proapoptotic function of AChE may have a role in suppressing tumor growth (Jiang and Zhang, 2008).

Given the involvement of AChE in apoptosis, it has been considered a potential target for therapeutic interventions. Strategies to modulate AChE activity or expression have been explored for cancer treatment (Lazarević-Pašti et al., 2017). For instance, in preclinical studies, AChE inhibitors or agents that enhance AChE expression have shown promise in promoting apoptosis and inhibiting tumor growth (Lazarević-Pašti et al., 2017; Khan et al., 2018; Recanatini and Valenti, 2004).

4. AChE in oxidative stress and inflammation

The AChE pathway has been implicated in oxidative stress, an imbalance between reactive oxygen species (ROS) production and antioxidant defenses. Oxidative stress occurs when there is an excess of ROS, including free radicals such as superoxide anions (O^{2-}), hydroxyl radicals (OH·), and reactive nonradical species such as hydrogen peroxide (H_2O_2). ROS can be produced during normal cellular metabolism or as a response to various stressors, and they can cause damage to cellular components such as DNA, proteins, and lipids. AChE can modulate oxidative stress through different mechanisms. AChE has been shown to possess antioxidant properties and can directly scavenge ROS (Zhang, 2005). It has been observed to have free radical scavenging activity, particularly against hydroxyl radicals. It can also protect against oxidative damage by reducing ROS levels and maintaining redox balance (Santi et al., 2011). AChE activity can influence cholinergic signaling and the activation of muscarinic and nicotinic receptors. Cholinergic signaling has been implicated in modulating oxidative stress responses. Activation of cholinergic receptors can regulate antioxidant enzyme activity and modulate cellular redox status. It plays a role in the cholinergic antiinflammatory pathway, which involves the modulation of inflammation and immune responses. Activation of cholinergic receptors by ACh can inhibit the release of proinflammatory cytokines and reduce oxidative stress associated with inflammation.

Oxidative stress can also affect the function of AChE. Increased ROS levels can lead to oxidative modification of AChE, resulting in decreased enzymatic activity and altered cholinergic signaling. This oxidative damage to AChE can further disrupt the balance of cholinergic signaling and contribute to oxidative stress−associated pathologies (Ferreira et al., 2015).

AChE has also been implicated in regulating inflammatory processes. Inflammatory responses involve the activation of immune cells, the release of inflammatory mediators, and the recruitment of immune cells to the site of inflammation. AChE has been found to have antiinflammatory properties by modulating the activity of immune cells and inflammatory mediators. It can inhibit the production of proinflammatory cytokines and chemokines, thereby suppressing the inflammatory response. AChE can also interact with immune cells, such as macrophages, to regulate their function and inflammatory activity (Liu et al., 2020). In the context of neuroinflammatory diseases, such as Alzheimer's disease, AChE has received significant attention. In neuroinflammation, AChE can contribute to the inflammatory process through multiple mechanisms. It can promote the activation of microglial cells, the immune cells of the central nervous system, leading to the release of proinflammatory substances. AChE can also modulate the activity of ACh receptors on immune cells, influencing their responsiveness to cholinergic signals and subsequent immune responses (Rosas-Ballina and Tracey, 2009).

The involvement of AChE in oxidative stress and inflammation has prompted investigations into its potential as a therapeutic target. Developing drugs that selectively modulate AChE activity or expression may offer opportunities for treating conditions associated with oxidative stress and inflammation (Ezoulin et al., 2007; Lazarević-Pašti et al., 2017). For example, AChE inhibitors have been explored as potential antiinflammatory agents in various disease models. The precise mechanisms and roles of AChE in oxidative stress and inflammation are still areas of active research.

5. AChE in lung cancer

Lung cancer is a prevalent and highly lethal form of cancer that originates in the lungs. It arises when abnormal lung cells rapidly multiply, forming tumors, and has the potential to metastasize to other parts of the body. Lung cancer can be broadly classified into two primary types: non−small-cell lung cancer (NSCLC) and small-cell lung cancer (SCLC). NSCLC accounts for most cases and encompasses subtypes such as adenocarcinoma, squamous cell carcinoma, and large cell carcinoma. At the same time, though less common, SCLC is known for its aggressive nature and faster growth and spread (Milankovic and Lazarevic-Pasti, 2021).

Lu et al. discovered that AChE exhibits abnormal expression in NSCLC and is functionally targeted by miR-212, a highly conserved molecule throughout evolution. The findings revealed that miR-212 negatively regulated AChE expression in laboratory settings and showed an inverse correlation between AChE levels and miR-212 expression in living organisms. Moreover, during cisplatin-induced apoptosis in NSCLC, AChE levels increased. In contrast, miR-212 levels decreased, indicating the role of AChE as a proapoptotic gene, promoting cell death. The study also demonstrated that upregulating AChE suppressed tumor growth in mice without functional immune systems. On the other hand, higher levels of

miR-212 protected against the apoptotic effects of AChE silencing in response to cisplatin-induced apoptosis. Restoring miR-212-resistant synaptic AChE expression inhibited the antiapoptotic function of miR-212. Overall, the research suggests that miR-212 has an antiapoptotic effect by directly repressing synaptic AChE expression in NSCLC, highlighting the potential role of synaptic AChE as a tumor suppressor regulated by miR-212 in NSCLC (Lu et al., 2013).

Exogenous nicotine and endogenous ACh binding to specific receptors promote the growth of both SCLC and NSCLC. Squamous cell lung carcinomas (SCC) exhibit elevated levels of certain nAChR, mRNA, and increased ACh levels, while cholinesterase mRNAs are reduced, resulting in enhanced cholinergic signaling. Nicotine exposure further enhances cholinergic signaling in SCC by increasing ACh secretion, elevating nAChR expression, and stimulating cell proliferation. To counteract the proliferative effects of nicotine and ACh on lung cancer growth, M3 mAChR antagonists have been explored as potential therapeutic agents. Notably, the M3-selective muscarinic antagonist darifenacin effectively blocked nicotine-stimulated cell growth in vitro and hindered tumor growth in vivo. This study emphasizes the significance of cholinergic signaling in the development and progression of SCC and highlights the potential of targeting this pathway for therapeutic interventions (Song et al., 2008).

Nicotine, a potent addictive component of cigarettes, stimulates lung cancer proliferation by acting on the α7-nicotinic acetylcholine receptor (α7-nAChR) subtype. This study investigates the impact of nicotine exposure on α7-nAChR levels in SCC, both in laboratory settings and living organisms. The findings reveal that nicotine increases α7-nAChR levels in human SCC cell lines and tumors, with upregulation dependent on the presence of GATA4 and GATA6 transcription factors. Clinical relevance is emphasized, as SCC-L patients typically have a smoking history before cancer diagnosis. It leads to continuous nicotine exposure and potential upregulation of α7-nAChRs, promoting tumor growth and progression. Implications extend to SCC patients exposed to nicotine through second-hand smoke, electronic cigarettes, or nicotine replacement therapies for smoking cessation. The study underscores the significance of understanding the molecular pathways driven by nicotine in lung cancer development and progression, offering potential targets for therapeutic interventions (Brown et al., 2013).

In the research conducted by Song et al., the primary objective was to investigate the potential of disrupting autocrine muscarinic cholinergic signaling to hinder the growth of SCLC. The results revealed that the M3 subtype of mAChR (M3R) significantly mediated these effects. Furthermore, it was evident that autocrine ACh secretion was responsible for stimulating MAPK and Akt phosphorylation, as M3R antagonists and M3R siRNAs alone reduced basal levels of MAPK and Akt phosphorylation in SCLC cell lines. Notably, when treated with M3R antagonists, the SCLC cells exhibited growth inhibition in laboratory settings (in vitro) and living organisms (in vivo), particularly in nude mice with tumor xenografts. Additionally, the M3R antagonists were found to decrease MAPK phosphorylation in the tumors in vivo. Immunohistochemical staining of SCLC and other cancer types revealed frequent coexpression of ACh and M3R. Based on these significant findings, the study suggests that M3R antagonists could potentially serve as valuable adjuvants in the treatment of SCLC and other cancer types as well (Song et al., 2007).

Song et al. investigated whether SCLC cells possess an autocrine cholinergic loop, producing and responding to ACh. The research involved examining a panel of SCLC cell lines for components involved in cholinergic signaling, such as ChAT, VAChT, and various nAChRs and mAChRs. Their findings demonstrate that SCLC cells can synthesize, secrete, and respond to ACh, suggesting the existence of an autocrine cholinergic loop that stimulates the growth of SCLC cells. This newly identified autocrine loop presents a potential target for therapeutic intervention in SCLC (Song et al., 2003).

6. AChE in prostate cancer

Prostate cancer is a common cancer in men, particularly in older age groups. Several studies have shed light on different aspects of prostate cancer: Jin-Chun et al. found that the expression of α5-nAChR is involved in prostate cancer cell proliferation. Silencing α5-nAChR inhibited the growth of DU145 prostate cancer cells but had minimal impact on PC3 prostate cancer cells. It highlights the significant role of α5-nAChR in promoting cell proliferation in specific prostate cancer cells and suggests its potential as a biomarker for prognosis and as a target for therapeutic interventions (Qi et al., 2020). Asain et al. focused on the molecular mechanisms and the impact of muscarinic acetylcholine receptor M1 (CHRM1) on prostate cancer migration and invasion. They discovered increased CHRM1 expression in early-stage prostate cancer tissues. The use of a CHRM1 antagonist hindered migration and invasion, while an agonist promoted these processes in prostate cancer cells. It suggests the involvement of CHRM1 and the hedgehog signaling pathway in prostate cancer migration and invasion, implying a potential interplay between muscarinic and hedgehog signaling (Yin et al., 2018). Battisti et al. investigated the activities of AChE and butyrylcholinesterase (BuChE) in patients with prostate cancer. They found lower AChE and BuChE activities in prostate cancer patients compared with the control group. The modulation of these activities varied depending on factors such as the Gleason score and the presence or absence of metastasis. Decreased cholinesterase activities were observed in advanced prostate cancer cases, suggesting a potential association between reduced cholinesterase activity and elevated ACh levels. The study also observed biochemical alterations and changes in liver enzyme activities in these patients (Battisti et al., 2012).

These studies collectively contribute to our understanding of prostate cancer and provide insights into potential biomarkers, therapeutic targets, and alterations in cholinesterase activities and biochemical parameters associated with the disease.

7. AChE in leukemia

Leukemia, a cancer affecting white blood cells, can be classified as lymphocytic or myeloblastic and further categorized as chronic or acute based on disease progression. Acute lymphoblastic leukemia (ALL) is characterized by the rapid multiplication of lymphoblasts in the bone marrow (Chang et al., 2021). A study on newly diagnosed ALL patients and those at different treatment stages revealed AChE activity changes. AChE activity in total blood

increased in the newly diagnosed group but decreased during remission induction and maintenance. Lymphocyte AChE activity showed similar patterns. Antineoplastic drugs cytarabine and methotrexate had differential effects on AChE activity. These findings suggest the involvement of the lymphocyte cholinergic system in ALL and its potential impact on hematological diseases and immune function regulation (Battisti et al., 2009).

The expression of cholinergic receptors, α7-nAChR and M3-mAChR, was investigated in the bone marrow or peripheral blood of healthy individuals and patients with leukemia. Leukemia patients generally had lower levels of α7-nAChR compared with the control group, with T-ALL showing the lowest expression. M3-mAChR expression varied among leukemia subtypes, with CML exhibiting the highest levels. These findings suggest cholinergic receptors as potential prognostic markers and therapeutic targets for leukemia treatment (Suriyo et al., 2019). Cholinergic signaling plays a role in leukemia, particularly chronic myeloid leukemia (CML). The study by Aydin et al. explored the effects of cholinergic agonists and antagonists on K562 cells derived from CML patients. The findings revealed that undifferentiated K562 cells exhibited increased AChE activity in response to ACh. However, high concentrations of ACh reduced AChE activity in erythroid-differentiated cells, and ACh did not increase AChE activity in megakaryocyte-differentiated cells. The study also investigated the effects of AChE inhibitors and observed that they did not increase AChE activity in a concentration-dependent manner. Furthermore, inhibiting choline uptake did not increase AChE activity in differentiated cells. The study suggests the involvement of cholinergic signaling in leukemia, highlighting the potential impact of ACh on cell growth and differentiation in different stages of leukemia development (Aydin et al., 2018).

8. AChE in breast and ovarian cancer

Breast cancer is a type of cancer that forms in the cells of the breast. It is one of the most common cancers among women globally, but it can also occur in men, although it is much rarer. Breast cancer usually originates in the breast's milk ducts (ductal carcinoma) or milk-producing glands (lobular carcinoma) (Sharma et al., 2010). Ovarian cancer is a type of cancer that originates in the ovaries, which are the female reproductive organs responsible for producing eggs and hormones. It is the most deadly gynecological cancer and the fifth leading cause of cancer-related deaths among women (Kurman and Shih, 2010). Paydar et al. conducted a study to evaluate the activity of AChE and arylesterase (ARE), as well as levels of malondialdehyde (MDA) and total antioxidant capacity (TAC) in the plasma of patients with breast cancer and benign breast diseases. The study included three groups: malignant breast tumor (MBT), benign breast disease (BBD), and a control group. The results showed that AChE and ARE activity were lower in the MBT and BBD groups than in the control group. Additionally, MDA levels were higher in the MBT and BBD groups, indicating increased oxidative stress. The TAC levels did not show significant changes among the groups. The study suggests that AChE activity could serve as a biomarker for studying oxidative stress in breast disease patients (Paydar et al., 2018). Another study focused on examining the expression of AChE during apoptosis induced by cisplatin in the MCF-7 human breast cancer cell line. The researchers treated MCF-7 cells with different concentrations

and durations of cisplatin to assess its apoptotic effect. They observed an increase in AChE expression in cisplatin-induced apoptotic cells over time. Furthermore, the study investigated the role of p53, a tumor suppressor protein, in apoptosis by treating cells with cisplatin alone or with p53 siRNA. The group with p53 siRNA showed a higher percentage of live/surviving cells and a lower percentage of apoptotic cells compared with the cisplatin-alone group. The study concludes that AChE levels were increased in MCF-7 cells following cisplatin treatment and that AChE may act as a downstream component of the p53 pathway, contributing to the induction of apoptosis during chemotherapy (Ye et al., 2015). Bernardi et al. studied amplification and deletion in AChE and BuChE genes in breast cancer patients. They used polymerase chain reaction (PCR) to analyze peripheral blood samples from 42 patients. The study found a higher frequency of deletions than amplifications in both BuChE and AChE genes in tumor tissues. There were positive correlations between AChE gene amplification/deletion and tumor size and a negative correlation between AChE gene amplification/deletion and ERBB2 proto-oncogene amplification. The study suggests a potential relationship between cholinesterase gene alterations and breast cancer characteristics (Bernardi et al., 2010). Researchers investigated the therapeutic potential of the ethanolic extract of *Cephalaria elazigensis* var. *purpurea* (CE) in the MCF-7 breast cancer cell line. They evaluated its antiproliferative effect, impact on colony formation and cell migration, and proapoptotic capacity. The IC_{50} value for CE was determined, and the extract exhibited a significant increase in apoptotic cell population with increasing concentration. The study also assessed the antioxidant activity of CE using various assays and observed its inhibitory effect on AChE and BuChE enzymes. Molecular docking analysis revealed the interaction of specific compounds with AChE and BuChE. These findings suggest the potential of CE as a therapeutic agent for breast cancer treatment (Erdoğan et al., 2023). A study by Motamed-Khorasaini et al. investigated gene expression changes in ovarian cancer cells and ovarian surface epithelial cells in response to androgen exposure. They identified 17 genes that showed differential expression, including BACH2 and AChE, which were upregulated in ovarian cancer cells. Further analysis revealed their association with clinical outcomes, with high levels of cytoplasmic AChE associated with decreased survival and nuclear BACH2 staining correlated with decreased time to disease recurrence. These findings suggest the potential of these genes as prognostic indicators and targets for further research in ovarian cancer (Motamed-Khorasani et al., 2007).

9. AChE in gastric and hepatocellular cancer

Gastric cancer (GC), also known as stomach cancer, is a malignant tumor that develops in the stomach lining. Hepatocellular carcinoma (HCC), also known as hepatoma, is the most common type of primary liver cancer. It originates in the hepatocytes, the liver's main cells. Xu et al. examined the expression of AChE in GC tissues and adjacent noncancerous tissues. Higher levels of AChE expression were associated with longer survival time in GC patients. Treatment with adenoviral vectors delivering AChE (Ad.AChE and ZD55-AChE) inhibited GC cell growth and induced apoptosis. ZD55-AChE, in particular, showed higher antitumor efficacy and suppressed the growth of GC stem cells. The study suggests that ZD55-AChE

could be a potential therapeutic approach for human GC (Xu et al., 2014). Another study investigated changes in gene expression of serotonin receptors, AChE, and monoamine oxidase A in GC. Higher levels of gene transcripts for serotonin receptors (5-HTR2A, 5-HTR2B, 5-HTR3A, 5-HTR7) and AChE were observed in the peripheral blood of GC patients compared with healthy individuals. In tumor tissues, increased expression of 5-HTR2B and 5-HTR3A genes and decreased expression of AChE were found compared to adjacent noncancerous tissues. These findings suggest a potential role of serotonin receptors and AChE in GC and highlight the possibility of targeting these factors for therapeutic and preventive approaches (Abedini et al., 2023). Researchers also investigated the association between the M3 receptor and ACh in GC. The M3 receptor was significantly overexpressed in GC tissues compared with noncancerous tissues. The elevated expression of M3 was associated with cancer stage and lymph node metastasis, suggesting its potential role in GC development. Treatment with exogenous ACh promoted the proliferation of GC cells, an effect blocked by selective M3 receptor antagonists. Inhibition of the M3 receptor led to a suppression of cell proliferation and induction of apoptosis. The study also observed the secretion of ACh by GC cells and the coexpression of ChAT and M3 in human GC tissues and cell lines. These findings suggest that M3 antagonists could potentially be adjuvants for GC therapies (Wang et al., 2016). Yang et al. investigated the role of ACh in promoting GC cell invasion, migration, and epithelial–mesenchymal transition (EMT) via the M3 receptor/AMPK/MACC1 signaling pathway. ACh was found to increase the number of invasive and migrating cells and decrease the expression of E-cadherin, indicating the promotion of invasion, migration, and EMT. Blockade of the M3 receptor inhibited GC cell invasion, migration, and EMT, even without exogenous ACh. The study also revealed that ACh upregulated MACC1 in GC cells, and MACC1 knockdown attenuated the effects of ACh on GC cells. AMPK was identified as an intermediate signal between ACh and MACC1, suggesting that ACh acts via M3 receptors and AMPK to regulate MACC1 and promote GC cell invasion and migration. These findings provide insights into GC growth, metastasis mechanisms, and potential treatment approaches (Yang et al., 2016). Yu et al. investigated the role of ACh in GC cell proliferation. They found that GC cells can synthesize and release ACh via the ChAT protein. Exogenous ACh promoted cell proliferation in GC cells and activated the ERK1/2 and AKT signaling pathways. The M3 receptor was identified as the specific muscarinic receptor subtype involved in ACh-induced cell proliferation. ACh also activated the EGFR pathway through the M3 receptor, further promoting cell proliferation. Inhibition of the M3 receptor or the EGFR pathway attenuated ACh-induced cell proliferation. In vivo experiments using M3 antagonists demonstrated their interference with GC growth. Additionally, M3 antagonists increased the sensitivity of GC cells to chemotherapy drugs and induced apoptosis-related protein expression. These findings suggest that targeting the M3 receptor or combining M3 antagonists with chemotherapy drugs may have therapeutic implications for GC treatment (Yu et al., 2017). The effect of AChE on the proliferation of HCC cells and the formation of spheroids was investigated. The researchers observed increased AChE content in high-density HCC cell cultures. Changes in cell cycle regulators, including cyclin D1, p27, p53, and p21, were also detected. The study examined AChE activity and protein levels to determine its potential involvement in cell cycle control. The results showed a higher level of AChE protein but reduced AChE activity in high-density HCC cells. This inverse relationship suggests a potential connection between AChE, cell growth, and reduced AChE activity

in hepatocellular carcinoma. Spheroid formation was observed, and the number of spheroids increased in cells exposed to the AChE inhibitor and in cells cultured with ACh and the inhibitor. The findings support the tumor suppressor role of AChE and its potential as a therapeutic target in HCC (Pérez-Aguilar et al., 2015). Zhao et al. examined the role of AChE in HCC. They observed a significant reduction in AChE expression in HCC tissues, and low AChE expression was associated with tumor aggressiveness, increased risk of cancer recurrence, and poor survival rates. AChE was found to suppress HCC cell growth and proliferation in vitro and in vivo. Extracellular AChE inhibited cell proliferation by degrading ACh in the culture medium. Increased AChE expression led to the inactivation of MAPK and PI3K/Akt signaling pathways and the activation of GSK3b, promoting cell apoptosis. Additionally, AChE enhanced the sensitivity of HCC cells to chemotherapy drugs. These findings suggest that AChE plays a role in suppressing HCC and can be targeted to enhance chemosensitivity in HCC treatment (Zhao et al., 2011).

10. AChE in brain tumor

A brain tumor refers to an abnormal growth of cells within the brain. These tumors can be benign (noncancerous) or malignant (cancerous). Obukhova et al. investigated the clinical significance of analyzing AChE activity as a marker for gliomas. They evaluated AChE activity in tumor tissue and blood samples from patients with primary brain tumors. The results showed a significant decrease in AChE activity in both tumor tissue and blood, and this decrease correlated with the increasing degree of tumor anaplasia. Bioinformatic analysis revealed associations between AChE and proteins involved in the PI3K-AKT and Notch signaling pathways. These findings provide new insights into the mechanisms of glioma formation and may contribute to identifying diagnostic markers for brain tumors (Obukhova et al., 2021). Oligodendrocyte precursor cells (OPCs) are considered the cells of origin for gliomas, and ACh has been shown to maintain the primitive state of normal OPCs through mAChRs. In their study, the researchers analyzed RNASeq data from glioblastoma patients and observed high expression of the CHRM3 gene in OPC-like cells of the proneural glioma subtype. They also found that pharmacological inhibition of mAChRs in patient-derived glioma grafts effectively prevented tumor reinitiation. These findings suggest that the cholinergic microenvironment plays a role in maintaining glioma stem cells (GSCs) similar to normal OPCs, and mAChR antagonists could be repurposed as potential treatments for glioma (Anand et al., 2023). In a study on brain tumors, the activity of AChE and BuChE was assessed, and their relationship with tumor cell growth was examined. The researchers analyzed 30 brain tumor samples and observed the coexistence of AChE and BuChE in all samples. Different types of brain tumors showed variations in AChE and BuChE activity. Type II and III gliomas displayed moderate AChE and BuChE activity with slow or moderate cell growth. In contrast, type IV gliomas showed high AChE and BuChE activity with rapid cell growth. Meningiomas exhibited high AChE and BuChE activity, with varying cell growth rates. Oligodendrogliomas showed moderate AChE and BuChE activity with slow cell growth, while craniopharyngiomas had high AChE activity, low BuChE activity, and slow cell growth. These results indicate that the presence and activity of AChE and BuChE affect cell proliferation in brain tumors (Barbosa et al., 2001).

The expression and functional role of the M2 receptor in glioblastoma was investigated. Researchers found that M2 receptors were expressed as transcripts and proteins in glioblastoma cell lines and human glioblastoma samples. Treatment with the M2 agonist arecaidine reduced cell proliferation in glioma cell lines, with the greatest effect observed at a concentration of 100 μM. Arecaidine also induced cell cycle arrest and apoptosis in glioma cell lines. Comparing the sensitivity of glioma cell lines to the M2 agonist arecaidine and the chemotherapy drug temozolomide (TMZ), both drugs showed effectiveness, but arecaidine had a lower IC50 value than TMZ. These findings suggest that the activation of the M2 receptor could serve as a new therapeutic target for glioblastoma treatment (Ferretti et al., 2013). Cristofaro et al. studied the effects of the hybrid muscarinic agonists P-6-Iper and N-8-Iper on the growth and survival of glioblastoma cancer cells (GSCs). The orthosteric muscarinic agonist arecaidine reduced proliferation and induced apoptosis in GSCs. The agonist N-8-Iper showed comparable effects to arecaidine, significantly reducing cell proliferation even at low doses. In contrast, P-6-Iper did not have significant effects on GSC growth. The inhibitory effects of N-8-Iper were reduced by the administration of the M2 receptor antagonist methoctramine or by M2 receptor destruction, indicating that M2 receptor activation mediated the inhibitory effects of N-8-Iper. N-8-Iper induced severe cell apoptosis and DNA damage in glioblastoma cells, suggesting its potential as a therapeutic option through M2 receptor activation (Cristofaro et al., 2018).

11. Modulators of AChE activity as a therapeutics in cancer treatment

Given the involvement of AChE cancer, it represents a promising therapeutic target for treating these conditions. While AChE inhibitors have been extensively researched as anti-AD drugs, there is a growing interest in exploring their potential as anticancer agents (Lazarević-Pašti et al., 2017).

11.1 Inhibitors of AChE activity as a therapeutics in cancer treatment

Certain AChE inhibitors have the potential to be effective therapeutics for preventing and treating cancers with elevated AChE activity. However, when considering AChE inhibitors as anticancer agents, it is important to bridge the gap between in vitro and in vivo drug effects, particularly regarding drug concentration. The study conducted by Russo et al. (2015) extensively discusses the approaches and challenges associated with integrating in vitro and in vivo data to establish the concentration–effect relationship of specific AChE inhibitors. Several approved chemotherapeutic agents were initially screened for their ability to inhibit AChE activity. One of these agents, cisplatin, is commonly used in the treatment of bladder, testicular, ovarian, and head and neck cancers. In human plasma, peak levels of cisplatin can range from 1 μg/mL to 10 mg/mL (equivalent to 3.3 and 33.3 mM, respectively) depending on the therapeutic regimen (Penta et al., 1983). Notably, this concentration range falls within the reported concentrations (0.5–7 mM) that inhibit AChE activity by 27%–82% (Kamal et al., 1999). A recent study by Ye et al. (2015) demonstrated that in MCF-7 breast cancer cells, cisplatin at 0.1 mM upregulates the expression of AChE, mediated by the p53 pathway. This

finding suggests a potential link between AChE expression enhancement and the activation of the p53 tumor suppressor during cell apoptosis, providing a basis for exploring new therapeutic approaches to cancer. However, further research is necessary before these results can be implemented in practical applications. The commonly used anticancer agents daunorubicin and doxorubicin are chemotherapeutic drugs that can negatively affect the prognosis of patients with acute lymphoblastic leukemia (ALL). A study assessing the apparent Michaelis constant and apparent maximal velocity revealed that ALL is characterized by decreased AChE activity, which is further reduced by the inhibitory effects of both drugs (Niazi et al., 2014). These findings suggest that caution is necessary when considering the use of AChE modulators for treating cancers with unknown levels of AChE activity. It is important to note that therapeutic levels of cancer drugs, in some cases, do not reach the concentrations required in vitro to inhibit AChE activity. However, certain therapeutic doses of the drugs mentioned before can indeed impact AChE activity, potentially opening avenues for therapeutic strategies that effectively manage both cancer and Alzheimer's disease (AD) or, at the very least, prevent their worsening. Earlier research on metal complexes utilized for cancer treatment demonstrated that metal porphyrins, metal amine complexes, and certain cationic ruthenium polypyridyl complexes can effectively inhibit AChE activity (Vyas et al., 2014). Recent studies specifically focusing on ruthenium(II) polypyridyl complexes revealed their strong inhibitory activity against AChE (with IC_{50} values of 9.23 and 0.30 μM, respectively) but limited potential as anticancer agents (Vyas et al., 2014). However, our recent investigations involving Ru(II)−N alkyl phenothiazine complexes, namely chlorpromazine hydrochloride and trifluoperazine dihydrochloride, have demonstrated significant AChE inhibitory activity (unpublished data) and notable anticancer effects against human breast carcinoma and human colon adenocarcinoma cell lines (with cytotoxicity expressed as IC_{50} values below 25 μM after 48 h of treatment) (Krstić et al., 2010).

Donepezil, a commonly used AChE inhibitor, exhibits potent inhibitory activity against AChE in vitro, with an IC50 value of 0.015 μM (Hyatt et al., 2005). Studies have shown that concentrations achieved in patients after donepezil treatment (ranging from 0.05 to 0.16 μM) fall within the range required to inhibit 50% of AChE activity (Russo et al., 2015). Donepezil has been found to induce apoptosis in HL-60 human promyelocytic leukemia cells by triggering mitochondrial membrane potential loss, cytochrome-c release into the cytosol, and activation of caspases-8, -9, and -3, mediated by alterations in the expression of Bcl-2 family proteins (Ki et al., 2010). Its cytotoxicity expressed as IC50 ranged from 99.61 to 200 μM. Given that increased AChE expression has been observed in patients undergoing long-term donepezil therapy (Kračmarová et al., 2015), it can be inferred that the upregulated AChE might contribute to apoptosis in cancer cells treated with donepezil.

Imidazolium salts derived from the reaction between 1-alkyl imidazole and alkyl halides have shown the ability to inhibit AChE and human carbonic anhydrases (hCAs) I and II. These salts exhibit promising potential in treating various conditions, including Alzheimer's disease, epilepsy, glaucoma, and leukemia. The study highlights the therapeutic implications of imidazolium salts as inhibitors of AChE and hCAs (Yiğit et al., 2022).

Numerous bioactive compounds derived from natural sources possess anti-AChE activities, although limited clinical data regarding their anticancer effects are available. However, certain natural compounds demonstrate dual properties of both anti-AChE and anticancer

activities, providing a foundation for potential future therapeutics targeting cancer and AD through structural modifications and dosage optimization. For instance, berberine chloride, an isoquinoline alkaloid obtained from plants of the Berberidaceae family, exhibits inhibitory effects against AChE with an IC_{50} value of 0.44 μM. Docking studies have revealed that berberine forms hydrophobic interactions with specific residues in AChE, leading to strong binding affinity and inhibitory effects (Perneczky et al., 2012). Berberine chloride has also shown potential to induce apoptosis by modulating p53 expression in breast, liver, and colon cancer cell lines, with reported cytotoxicity expressed as IC_{50} values of 1.95, 1.73, and 1.83 μg/mL, respectively, after 72 h of incubation (Abd El-Wahab et al., 2013). Moreover, several protoberberine alkaloids, including palmatine, jateorizine, coptisine, and epiberberine derived from *Coptis chinensis*, are known to be effective AChE inhibitors, with IC_{50} values ranging from 0.51 to 1.07 μM. These alkaloids have also been reported as potential anticancer agents. Extracts from *C. chinensis* exhibit strong cytotoxicity against the hepatocellular carcinoma cell line Mwd HCC97L, with an IC_{50} value of 170 μM after 72 h of treatment (To et al., 2012). Another aporphine alkaloid, taspine, derived from plants of the Magnoliaceae family, has shown stronger inhibitory activity against AChE (IC_{50} 0.33 μM) compared with galantamine (IC_{50} 3.2 μM) (Murray et al., 2013) Taspine has also demonstrated its potential to induce apoptosis in breast cancer cells, with cytotoxicity expressed as IC_{50} of 1.69 μM (Zhan et al., 2011). Similarly, kokusaginine, one of the significant furoquinoline alkaloids from the Rutaceae family, exhibits AChE inhibitory activity (IC_{50} 46 μM) and has been reported to possess cytotoxic effects against certain leukemias, breast, colon, glioblastoma, and hepatocellular cell lines (with IC_{50} values ranging from 44.56 to 119.88 μM). In certain leukemia cell lines, kokusaginine appears to be more effective as an anticancer agent than doxorubicin (Adamska-Szewczyk et al., 2016; Sandjo et al., 2014).

Nonalkaloid AChE inhibitors, such as the flavonoids isoorientin and isovitexin (with IC_{50} values of 26.8 and 36.4 μM, respectively), derived from plants of the Iridaceae family, exhibit moderate anticancer activity against larynx, cervix, liver, and breast cancer cell lines (with IC_{50} values ranging from 18.5 to 32.30 μg/mL) (Farid et al., 2015). Moreover, the monoterpenes α-pinene and camphor are known for their potent anti-AChE properties (with IC_{50} values of 0.96 mM and 21.43 μM, respectively) (Farag et al., 2016; Zarrad et al., 2017) and have been reported to possess anticancer activities. α-Pinene, for example, has shown inhibitory effects on BEL-7402 hepatocellular carcinoma cells, with a cell growth inhibition rate of 79.3% when applied at a concentration of 8 mg/L for 72 h. Furthermore, α-pinene has demonstrated significant suppression of tumor cell growth in tumor-bearing mice (Chen et al., 2015).

Recent research has highlighted the potential of marine invertebrates as valuable sources of bioactive compounds. Polymeric alkylpyridinium salts derived from the marine sponge *Haliclona sarai* have demonstrated mixed reversible inhibition of AChE and have shown promise as chemotherapeutic agents. These compounds have been tested on human lung adenocarcinoma epithelial cell lines, with an EC50 of 4.41 nM, as well as on primary NSCLC cells, with an EC50 of 0.36 nM, both in vitro and in vivo (Garaventa et al., 2010; Sepčić et al., 1998). The authors of the study proposed that the inhibition of AChE by these compounds prevents the removal of ACh from the receptor, preventing ACh overflow and promoting apoptosis in cancer cells (Garaventa et al., 2010).

11.2 Enhancers of AChE activity as a therapeutics in cancer treatment

In most cancers, there is impaired AChE activity, leading to excessive production of ACh and the overactivation of muscarinic and nicotinic receptors, which promotes cancer cell proliferation (Xi et al., 2015). However, exceptions exist, such as in human glioblastoma cells, where ACh inhibits malignant growth by activating M2 muscarinic receptors (Ferretti et al., 2013). Recent reports have discussed the use of AChE protein in cancer therapy, specifically for hepatocellular carcinoma and gastric cancer (Xu et al., 2014; Zhao et al., 2011). Studies have shown that recombinant AChE protein and increased expression of AChE significantly inhibit the growth of hepatocellular carcinoma cells and sensitize tumor cells to anticancer drugs like etoposide, a DNA topoisomerase II inhibitor. Additionally, AChE delivered through an oncolytic adenoviral vector (ZD55-AChE) induces apoptosis in gastric cancer cells by activating the mitochondrial apoptotic pathway. These findings suggest that applying AChE enhancers may be a promising therapeutic strategy for treating cancers with downregulated AChE activity. Earlier studies by Deng et al. demonstrated that etoposide and excisanin A increase AChE levels in the colon cancer cell line SW620 through the c-Jun N-terminal kinase (JNK)/c-Jun pathway. The IC_{50} value of etoposide was 43.05 μg/mL (deng et al., 2006), which falls within the range of peak plasma concentrations achieved in patients. Plasma concentrations of etoposide have been reported in the range of 0.78−116.5 μg/mL (equivalent to 1.32−197.94 μM) (Hande, 1996; Tazawa et al., 2016), depending on the therapeutic regimen. Similar effects of etoposide have also been observed in human colon cancer cells (HT-29), human brain tumor cells (TE671 and U373MG), human melanoma cells (SK-MEL-5), and Malme-3M human melanoma cells (Xi et al., 2015). Pilocarpine, a selective M1/M3 muscarinic ACh receptor agonist, was investigated for its efficacy in chronic myeloid leukemia (CML). The study examined the effects of pilocarpine on M3 receptor expression, AChE activity, IL-8 release response, and cell proliferation in K562 cells derived from CML patients. The results showed that pilocarpine inhibited the chronic proliferation of myeloid cells, increased expression levels of apoptotic proteins, and induced apoptosis through the increase in AChE activity. The study suggests that an M3-selective agonist-like pilocarpine may have therapeutic potential in CML by inhibiting cell proliferation (Kanlı et al., 2023). Although these drugs significantly increase the expression of AChE, the precise mechanisms underlying their interaction with the AChE enzyme itself have yet to be fully elucidated, to the best of our knowledge.

12. Future perspective and conclusion

The information provided in this chapter emphasizes the importance of evaluating AChE activity in various cancer types prior to initiating chemotherapy with AChE modulators. However, further extensive research is required to fully comprehend the intricate role of AChE in the pathways governing cell proliferation and cell death. As we look toward the future, several key areas require extensive investigation to advance our understanding of AChE's involvement in cancer progression and its potential as a therapeutic target.

1. Unraveling molecular mechanisms: Future research efforts should focus on elucidating the specific molecular mechanisms through which AChE influences cell proliferation and death pathways in different cancer contexts. Advanced technologies such as single-cell sequencing and proteomics will play pivotal roles in deciphering the complex interactions between AChE and cellular signaling networks.
2. AChE isoform specificity: Investigating the functional differences between various AChE isoforms and their contributions to cancer development will be crucial. Identifying isoform-specific roles can provide valuable insights into disease biology and guide the development of isoform-specific therapeutic agents.
3. Personalized treatment approaches: Individual AChE levels and isoform ratios may serve as potential biomarkers for predicting treatment response and patient outcomes. Future studies should focus on incorporating these factors into personalized treatment strategies to optimize therapeutic efficacy and minimize adverse effects.
4. Targeted therapies: The development of innovative, targeted agents that selectively modulate AChE activity in cancer cells holds tremendous promise. By exploiting AChE's role in cancer biology, researchers can create novel therapies that offer improved treatment outcomes and enhance the overall quality of life for cancer patients.
5. Cross-disciplinary collaborations: Future progress in understanding AChE's role in cancer requires collaborative efforts between oncologists, neuroscientists, biochemists, and other experts. Integrating knowledge from different fields will foster a comprehensive understanding of AChE's multifaceted functions in both cancer and neurodegenerative diseases.

In conclusion, the exploration of AChE activity in the context of cancer biology has opened new avenues of research with exciting potential. Evaluating AChE activity prior to chemotherapy with AChE modulators represents a crucial step in optimizing cancer treatment outcomes. However, this is just the beginning of a journey that promises to unravel the complex role of AChE in cell proliferation and death pathways. The future of AChE research lies in unraveling the intricate molecular mechanisms governing its functions, understanding the specificity of AChE isoforms in cancer contexts, and harnessing AChE-related biomarkers for personalized treatment approaches. Targeted therapies that capitalize on AChE's involvement in cancer hold great promise for revolutionizing cancer treatment strategies. Moreover, the implications of AChE research extend beyond oncology, with potential benefits for neurodegenerative diseases as well. By pushing the boundaries of scientific knowledge and fostering interdisciplinary collaborations, we can envision a future where specialized AChE-targeting agents are harnessed to combat both cancer and neurodegeneration effectively. As we embrace the future with optimism, it is essential to continue supporting and investing in AChE research. By doing so, we can move closer to translating these scientific discoveries into tangible benefits for patients, offering them improved treatment options and better prospects for long-term health and well-being. The journey ahead is promising, and with dedication and collaboration, we can unlock the full potential of AChE as a transformative force in cancer therapy and neurological medicine.

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CHAPTER

19

Therapeutics of animal-derived compounds in proteases-induced cancer

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1. Introduction

Protease inhibitors are an important class of molecules in biological systems known for their role in modulating protease activity. These inhibitors are critical for the regulation of proteolytic processes in organisms, which are essential for various physiological processes including digestion, blood coagulation, immune response, and cell signaling pathways (Drag and Salvesen, 2010). These inhibitors also play a central role in maintaining homeostasis by preventing uncontrolled protease activity that can lead to pathological conditions (Nieman, 2016). In recent years, research into natural sources of novel protease inhibitors has intensified, particularly in the field of drug discovery.

As previously mentioned, proteases play an important role in cancer progression by facilitating metastasis, angiogenesis, and evasion of the immune system (Vizovisek et al., 2021). The development of protease inhibitors as therapeutic agents represents a promising approach in oncology, with a focus on specificity and minimal side effects (Crawford et al., 2011). The search for novel inhibitors has focused on natural sources, including venomous animals, especially those that are not commonly studied. It has been demonstrated that these animals are a valuable source of distinctive and efficacious bioactive compounds. These include protease inhibitors, which have immense potential in various therapeutic areas. In particular, the venoms of marine and terrestrial species are being investigated for their novel components that target critical biological signaling pathways (Mourão and Schwartz, 2013). These venoms contain a complex mixture of proteins, peptides, and enzymes that

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have evolved to target specific molecular pathways in prey or predators. This diversity makes them a promising source for drug discovery, especially for the development of new therapeutics against cancer. This chapter will explore the potential of protease inhibitors from rare venomous animals in the context of cancer drug discovery. The interest in these inhibitors stems from their unique structural and functional properties, which may offer new opportunities for targeting cancer-related signaling pathways. Protease inhibitors from animal venoms have been shown to exhibit a range of biological activities, including antitumor effects, suggesting their potential application in cancer therapy (Li et al., 2018). This chapter provides an overview of protease inhibitors derived from animal venoms and highlights their structural features, mechanisms of action, and potential therapeutic applications in cancer treatment. By examining these critical aspects, the growing field of anticancer drug development will be enriched by gaining valuable insights into how these natural compounds could revolutionize the treatment of cancer.

2. Use of venom-derived compounds in medicine

Research into toxins in medicine has significantly increased our understanding of how these natural substances can be used for therapeutic purposes. In particular, protease inhibitors have attracted attention due to their central role in regulating proteolytic activities that are essential for a variety of physiological processes (Clemente et al., 2019). The balance of protease functions is critical for maintaining the integrity of various biological systems, including digestion, blood coagulation, immune responses, and cellular signaling. Research highlights the crucial role that these inhibitors play in maintaining proteolytic balance and emphasizes their importance in maintaining physiological harmony (Antalis et al., 2007; Oliveira et al., 2022; Almeida et al., 2022; Mohamed Abd El-Aziz et al., 2019).

In the context of cancer, proteases have been identified as key factors for tumor growth, invasion, and spread of cancer cells. These enzymes regulate growth factors and signaling receptors and alter the extracellular matrix, facilitating cell migration, matrix degradation, and promotion of tumor metastasis. The multifunctional role of proteases in cancer, including angiogenesis, cell communication, and metastasis (Kai et al., 2019; Park et al., 2020), underscores the urgent need for targeted interventions in these signaling pathways. Venomous animals, known in traditional medicine for their therapeutic potential, have proven to be valuable sources of bioactive molecules. Snakes, scorpions, and toads in particular produce venoms rich in proteins, peptides, and enzymes with significant biological effects. These venom components have been investigated for their applicability in the treatment of various diseases, especially cancer. The potential of venom-derived compounds in oncology lies in their distinctive properties, which can be harnessed to effectively target cancer cells (Vyas et al., 2013). Recent scientific advances have not only validated the traditional uses of venom-based substances but also expanded our understanding of their potential mechanisms of action and clinical applications. These advances have paved the way for the development of innovative treatment strategies and drug discovery initiatives. For example, the use of chlorotoxin, derived from scorpion venom, has shown promise for targeting glioma cells and offers a new approach for the treatment of brain tumors (Dardevet et al., 2015). In addition, captopril, a drug developed from the venom of the Brazilian pit viper, has become a

cornerstone in the treatment of hypertension and heart failure, illustrating the successful transition of venom-derived agents into regular pharmacotherapy (DiBianco, 1986). In addition, research into marine toxins has produced a new class of protease inhibitors that can be used in anticoagulation and cancer treatment. Compounds isolated from marine snails, such as conotoxins, have demonstrated the ability to modulate ion channels and neurotransmitter release, offering insights into new therapeutic avenues for pain management and neurological disorders (Mourão and Schwartz, 2013). As research unravels the complexity of venom-derived protease inhibitors and their interactions with biological systems, the potential of these compounds for medical science is becoming increasingly clear. The integration of multidisciplinary approaches combining biochemistry, pharmacology, and clinical research is essential for the translation of these natural substances into effective therapies. This ongoing research not only holds the promise of new treatments for difficult diseases such as cancer but also highlights the value of biodiversity and natural resources for improving human health.

3. Protease inhibitors from rare toxic marine species

The study of marine bioactive compounds, particularly protease inhibitors, has evolved into a dynamic field of research that utilizes the remarkable diversity of marine ecosystems to discover new therapeutics and industrial applications. The journey from initial discovery to practical application is both complex and fascinating, illustrating the potential that the ocean offers for improving human health and technology. In the fight against cancer, marine-derived protease inhibitors offer a promising avenue for the development of targeted therapies. Gallinamide A, a potent inhibitor of cathepsin L isolated from marine cyanobacteria, is an example of the targeted approach these compounds can offer. The work of Miller et al. (2014) has shown that by specifically inhibiting a protease involved in cancer progression, treatments can be developed that are both effective and have fewer side effects than conventional chemotherapy. This specificity not only increases therapeutic efficacy but also significantly reduces the toxicity profile of the treatment, making it more tolerable for patients. The neuroprotective effects of marine-derived compounds such as the Zoanthus Kunitz-like peptide ZoaKuz1 represent another promising area of application. The study by Liao et al. (2019), which demonstrated the potential of ZoaKuz1 in a zebrafish model of Parkinson's disease, suggests that similar compounds could be developed to treat a range of neurodegenerative diseases. These findings pave the way for novel treatments that could potentially halt or even reverse the progression of debilitating diseases such as Parkinson's, Alzheimer's, and ALS, giving hope to millions of patients and their families. Beyond their medical applications, marine protease inhibitors have demonstrated their potential in various industries. For example, the alkaline protease inhibitors identified by Ji et al. (2013) possess properties that make them detergent additives capable of functioning effectively in the high pH environment of detergents. This application not only highlights the versatility of marine compounds but also points to the sustainable and biodegradable nature of these substances, which represent an environmentally friendly alternative to synthetic chemicals. An example of the transition from marine toxins to clinically approved therapeutics is the development of ziconotide, derived from the venom of the cone snail. This peptide has revolutionized pain management by providing chronic pain patients with an effective nonopioid

analgesic option. The discovery of ziconotide underscores the potential of marine venoms as a source of new drugs with significant clinical impact, offering alternatives to opioid analgesics and the associated risks of dependence and side effects.

The path from discovery to application of bioactive marine compounds presents numerous challenges, including the sustainable sourcing of these materials, the need for extensive preclinical and clinical testing, and the development of cost-effective production methods. Advances in biotechnology, such as synthetic biology and marine bioprospecting, offer promising solutions to these challenges and enable the sustainable and efficient production of these compounds. In addition, the integration of computational methods such as molecular docking and virtual screening into traditional drug discovery processes can accelerate the identification of promising compounds (Karthikeyan et al., 2022). These technologies enable rapid screening of extensive libraries of marine natural products against a broad range of targets, identifying potential hits at a fraction of the time and cost of traditional screening methods. As research in this field progresses, collaboration between the various disciplines—marine biology, pharmacology, biochemistry, and biotechnology—will be crucial to unlock the full potential of marine biodiversity. The examples given here are just the tip of the iceberg, as the vast majority of marine organisms remain unexplored. Continued exploration and study of marine life promises the discovery of even more potent and specific bioactive compounds that offer new solutions to global health problems and beyond. The study of bioactive compounds from the ocean, particularly protease inhibitors, represents a frontier area of scientific research that offers tremendous potential for improving human health and advancing technology. The journey from the depths of the ocean to the pharmacy shelf is complex and requires innovation, collaboration, and perseverance (Karthikeyan et al., 2022). However, the successes achieved to date underscore the immense value of ocean biodiversity as a source of novel, lifesaving therapies and sustainable industrial solutions that inspire further exploration and discovery. Among the countless substances produced by marine organisms, protease inhibitors stand out due to their diverse therapeutic applications and mechanisms of action (Rotter et al., 2021). These compounds, often derived from the venom of marine animals known for their toxic production, such as sea anemones, cone snails, and jellyfish, offer promising opportunities for drug development and other applications. Kunitz-type protease inhibitors are an excellent example of the therapeutic potential of marine species. These inhibitors, characterized by their unique amino acid arrangement and stabilized by disulfide bridges, target serine proteases such as trypsin and chymotrypsin and are characterized by remarkable specificity and potency. These inhibitors, found in the venom of sea anemones and cone snails, demonstrate not only the molecular diversity of marine bioactive compounds but also their potential to block critical biological signaling pathways, such as those involving K+ channels, which are important for various physiological processes (García-Fernández et al., 2016; Hakim and Yang, 2016). The research of Mourão and Schwartz has decisively underlined their importance in the study of protease inhibitors in marine toxins (Mourão and Schwartz, 2013). Another interesting example is the venom of *Thalassophryne nattereri*, a small venomous fish whose venom inhibits the blood angiotensin-converting enzyme (ACE) in the blood and thus has properties that could potentially be used for the treatment of blood pressure and cardiovascular diseases. This toxin, which contains natterins and a lectin called Nattectin, exhibits inflammatory and necrotic properties and

offers a unique insight into the therapeutic and biochemical study of toxins (Lopes-Ferreira et al., 2014).

The advanced transcriptomic and proteomic studies on *Zoanthus natalensis* have revealed peptidotoxins, including neurotoxins and protease inhibitors. In particular, the Kunitz-like peptide ZoaKuz1, which is able to inhibit voltage-gated potassium channels and exert neuroprotective effects, suggests new possibilities in the treatment of neurological disorders such as Parkinson's disease and demonstrates the potential of marine compounds in the treatment of complex human diseases (Liao et al., 2019). The venom of *Protopalythoa variabilis*, a zoanthid species, **exhibits** a wide range of toxin-like polypeptides, including neurotoxins and hemorrhagic toxins, highlighting the diversity and evolutionary significance of transcripts associated with marine toxins (Huang et al., 2016). This diversity is also reflected in the broader spectrum of marine-derived compounds, such as marine alkaline protease inhibitors that show promise as detergent additives, gallinamide A for its selective inhibition of cysteine proteases, and marinostatin with its unique structure and function among serine protease inhibitors (Boudreau et al., 2019; Chen et al., 2019; Unno et al., 2021). The trypsin inhibitor is derived from the marine bacterium *Oceanimonas* sp. BPMS22 and is characterized by its competitive inhibition and anticoagulant properties. It has also shown anticancer potential, underlining the dual therapeutic role of such inhibitors. The molecular weight and mechanism of competitive inhibition make this inhibitor an interesting subject in the study of marine protease inhibitors (Jayaraman et al., 2022). These examples illustrate only a fraction of the bioactive compounds found in venomous marine animals and provide insight into the potential for new therapeutic agents and industrial applications. The unique structures and mechanisms of action of these compounds not only provide valuable insights into biological processes but also highlight the enormous potential of the marine environment as a source of novel bioactive compounds. Continued research and study of these compounds will undoubtedly uncover further applications in medicine and offer new strategies for treating cancer, neurological disorders, and more. The journey from the depths of the ocean to clinical and industrial applications represents a promising frontier in the search for innovative solutions to some of humanity's most pressing health challenges.

3.1 Empirical studies and findings on the use of protease inhibitors from marine toxins in cancer therapy

The exploration of protease inhibitors from marine toxins for cancer therapy is an emerging field of research that utilizes the unique properties of these compounds to develop new treatment strategies. The complexity and specificity of marine toxins makes them particularly attractive for interfering with the multifaceted mechanisms underlying cancer progression and metastasis. Empirical studies have begun to uncover the therapeutic potential of these marine-derived compounds, offering insights into their mechanisms of action and potential clinical applications. For example, the neuroprotective effect of ZoaKuz1, a peptide toxin from *Zoanthus natalensis*, suggests that it is relevant not only for neurodegenerative diseases but also for cancer, as some of the underlying mechanisms overlap between these diseases (Liao et al., 2019). This underlines the broad therapeutic potential of marine toxins beyond their original targets. Further research into the cardiovascular effects of compounds such as ACE and ECE from cone snail venom has opened new avenues for investigating their

role in cancer-related signaling pathways. Safavi-Hemami et al. (2015) discuss the implications of these findings and suggest that manipulation of such signaling pathways could influence cancer progression and metastasis, highlighting the interconnectedness of physiological systems and disease. Safavi-Hemami et al. (2015) and Barzkar et al. (2021) provide a comprehensive review of marine serine protease inhibitors, emphasizing their role in controlling processes critical to cancer metastasis, such as inflammation and immune responses. The targeting of these inhibitors to serine proteases involved in carcinogenesis makes them promising candidates for anticancer drugs and offers a more targeted approach to disrupting cancer pathways (Barzkar et al., 2021). One of the most promising compounds in this area is salinosporamide A, derived from marine bacteria, which has been shown to inhibit the proteasome, a key component in protein degradation in cells. The potent inhibitory effect of this compound and its selective targeting of cancer cells has led to it being tested in clinical trials for the treatment of multiple myeloma and other cancers (Gulder and Moore, 2010; Macherla et al., 2005), highlighting the translational potential of marine-derived inhibitors. Tasiamide F, which is derived from marine cyanobacteria, shows strong inhibition of cathepsins D and E, enzymes that play a role in carcinogenesis and immune regulation. This indicates the potential of tasiamide F and its analogs in cancer therapy and is indicative of a new class of anticancer drugs (Al-Awadhi et al., 2016). Research on marine *Streptomyces* sp. has revealed the production of dihydroeponemycin with remarkable proteasome inhibitory activity and efficacy against glioma cell lines. This discovery opens up potential therapeutic opportunities for brain tumors and demonstrates the versatility of marine toxins in combating various types of cancer (Furtado et al., 2021). In addition, the peptide trypsin inhibitor from *Oceanimonas* sp. BPMS22 exhibits significant anticoagulant and anticancer properties by inducing apoptotic effects in leukemia cell lines. This dual functionality underlines the potential of marine toxins as versatile therapeutics (Harish et al., 2021). Overall, these studies underline the enormous potential of protease inhibitors from marine toxins in the field of cancer therapy. They not only pave the way for new drug discovery and development strategies but also enable a deeper understanding of the molecular basis of cancer. Further research into these compounds is crucial for translating their unique biological activities into clinically relevant treatments that could revolutionize cancer therapy with marine-derived drugs.

4. Protease inhibitors from rare terrestrial venomous animals

In researching venom-derived protease inhibitors, the scientific community has discovered an even broader spectrum of compounds with unique biological activities and therapeutic potential. These inhibitors, derived from a variety of venomous organisms, have not only provided new insights into biochemical metabolic pathways but also paved the way for innovative drug development strategies. Kunitz protease inhibitors, commonly found in various venomous terrestrial animals such as snakes, scorpions, and spiders, are known for their targeted inhibition of serine proteases such as trypsin and chymotrypsin. These inhibitors, which usually consist of about 60 amino acids and are reinforced by three disulfide bridges, are characterized by their structural integrity and specificity. Their diverse molecular

functions, which go beyond enzyme inhibition and also include effects such as the blocking of potassium channels, underline their potential in various therapeutic areas (Mourão and Schwartz, 2013). In addition to the examples already mentioned, other representatives of Kunitz-type protease inhibitors have been identified, illustrating the great diversity within this group. For example, inhibitors from the venom of the Amazonian snake *Bothrops moojeni* have been shown to have the potential to modulate blood coagulation processes, suggesting applications in the treatment of thrombotic diseases (Gonçalves Machado et al., 2015). Research into these inhibitors shows that they are able to target specific coagulation factors, offering a more targeted approach to anticoagulant therapy than conventional treatments. Recent studies have identified new serine protease inhibitors in the venom of various spider species. These compounds, isolated for example from the Brazilian wandering spider (*Phoneutria nigriventer*), exhibit unique mechanisms of action, including inhibition of coagulation factors and potential antihypertensive effects. Their specificity and efficacy highlight the therapeutic potential of spider venoms as a source for the development of new cardiovascular drugs (Peigneur et al., 2018). Collinein-1, a serine protease from the venom of *Crotalus durissus collilineatus*, inhibits the cancer-relevant hEAG1 potassium channel. Its unique mechanism involves a large ligand that acts as an ion channel inhibitor. In addition, it exhibits fibrin(-ogen)olytic activity in mammals, further emphasizing its potential for therapeutic applications (Alves and Ferreira, 2022; Boldrini-França et al., 2015). Cysteine protease inhibitors from snake venom, as found in the venom of the King Cobra (*Ophiophagus hannah*), have been shown to inhibit key enzymes involved in immune responses and cell death pathways. These findings suggest potential applications in autoimmune diseases and conditions with excessive cell proliferation, such as cancer. The specificity of these inhibitors for cysteine proteases underscores the potential for the development of targeted therapies that minimize off-target effects (Danpaiboon et al., 2014). These cystatin-like cysteine protease inhibitors, particularly an isoform related to family 2 cystatins, are found in lizard snake venoms and show inhibitory effects on enzymes such as cathepsin L and papain, highlighting their potential for various therapeutic applications (Richards et al., 2011). Ascaris-type protease inhibitors (*Scorpiops jendeki*): The discovery of Ascaris-type protease inhibitors, such as SjAPI from the venom of *S. jendeki*, represents a significant milestone in the understanding of venom-derived protease inhibitors. With its unique Ascaris-type cysteine scaffold and five disulfide bridges, SjAPI exhibits dual inhibition against α-chymotrypsin and elastase. Its structural uniqueness and functional versatility make it a promising model for the development of new serine protease inhibitors with a wide range of therapeutic possibilities (Chen et al., 2013). SdPI from scorpion venom (*Lychas mucronatus*): SdPI, isolated from the venom of *L. mucronatus*, represents a new category within the Kunitz venom peptides. SdPI is characterized by a distinctive cysteine scaffold with three linked disulfide bridges and is a potent and thermally stable trypsin inhibitor. This discovery expands the understanding of the structural and functional diversity of Kunitz venom peptides and opens up new possibilities for their therapeutic use in research, particularly in the modulation of serine protease activities (Zhao et al., 2011).

Further research into bee venom has led to the discovery of other inhibitors with antiviral and antimicrobial properties. Compounds such as melittin have been studied for their ability to destroy viral envelopes and bacterial membranes, offering a promising avenue for the development of new antimicrobial and antiviral agents. This extends the application

potential of bee venom far beyond its anticoagulant and antiinflammatory properties. Serine protease inhibitor from bee venom (Bi-KTI): Bi-KTI from bee venom acts primarily as a plasmin inhibitor and is a potent antifibrinolytic agent. Its unique selectivity for plasmin as opposed to other coagulation enzymes such as factor Xa or thrombin indicates its potential in the treatment of diseases with excessive fibrinolysis. The specificity and efficacy of Bi-CTI in plasmin inhibition make it a candidate for clinical use, particularly in the treatment of bleeding disorders and related diseases (Choo et al., 2012). Scorpion venom continues to be a rich source of bioactive compounds, with recent discoveries of inhibitors with potential anticancer properties. For example, chlorotoxin isolated from the venom of the death scorpion (*Leiurus quinquestriatus*) has shown promise in targeting specific cancer cell lines and provides a basis for the development of targeted cancer therapies. This specificity toward malignant cells gives hope for treatments that have fewer side effects compared with conventional chemotherapy. MeKTT-1 from scorpion venom (*Mesobuthus eupeus*), MeKTT-1 is derived from the scorpion venom of *M. eupeus*, belongs to the Kunitz toxin family, and specifically inhibits trypsin. The ability of MeKTT-1 to prolong scorpion venom-induced pain behavior in animal models suggests a role in the venom's efficacy and its potential for pain therapies (Ma et al., 2016). Even lizards, often overlooked in venom studies, have contributed to the pool of protease inhibitors. The Gila monster (*Heloderma suspectum*) produces exendin-4, a substance that has been developed into a drug for the treatment of type 2 diabetes. This example underscores the potential of toxin-derived compounds to be converted into effective therapeutics for chronic diseases. These real-world examples illustrate the incredible diversity and potential of toxin-derived protease inhibitors as a source of new therapeutic agents. Each discovery not only contributes to our understanding of the complex biological functions of venoms but also opens up new avenues for drug discovery and development and highlights the untapped potential of venoms in the treatment of a wide range of human diseases. The ongoing exploration of these compounds promises to find even more innovative solutions to some of the most challenging medical problems and embodies the convergence between nature's ingenuity and man's scientific endeavors (Yap and Misuan, 2019). Each of these protease inhibitors is an example of the extraordinary diversity and specificity of venom-derived compounds. Their different structural and functional properties make them valuable models for the development of new therapeutics, especially for the treatment of cancer and other diseases in which protease activity plays a crucial role.

The study of protease inhibitors derived from venomous terrestrial animals not only deepens our knowledge of venom biology but also serves as fertile ground for the development of innovative therapeutic agents. The different structure and specialized mechanisms of action of the individual inhibitors underscore the remarkable potential of venom-derived substances for medical and pharmaceutical research. From influencing serine protease activity in pain management to affecting blood clotting and fibrinolysis, these inhibitors offer insights into novel approaches for treating a variety of health conditions. Their broad functional properties and complex structural details make them exemplary models for medical research, particularly in areas where existing treatment options are inadequate. Ongoing research into

these protease inhibitors will greatly enrich the field of therapeutic solutions by utilizing the unique biochemical resources of venomous terrestrial animals.

4.1 Empirical studies and findings on protease inhibitors from terrestrial toxins in cancer therapy

Terrestrial venomous animals such as snakes, scorpions, spiders, and certain insects produce a range of protease inhibitors that have evolved for various biological purposes, including defense and immobilization of prey. In particular, Kunitz-type protease inhibitors, which are widely distributed in these species, are known to inhibit serine proteases such as trypsin and chymotrypsin. These inhibitors are usually formed from amino acid chains reinforced by disulfide bridges, which gives them structural stability and functional specificity (Mourão and Schwartz, 2013). An interesting example is found in the venom of the fish *Scorpaena plumieri*, which contains protease inhibitors among other toxins. These inhibitors exhibit proteolytic and antimicrobial activities and are associated with cardiovascular, inflammatory, hemolytic, and nociceptive effects following envenomization (Borges et al., 2018). Another case concerns Ascaris-type protease inhibitors in scorpion venom. In contrast to Kunitz-type inhibitors, peptides such as SjAPI from the scorpion *S. jendeki* have a classical Ascaris-type cysteine scaffold. SjAPI is characterized by its dual inhibitory function against α-chymotrypsin and elastase, which underlines its potential as a model for new serine protease inhibitors (Ding et al., 2015). Snake venom−derived protease inhibitors have shown promise in cancer research. Kunitz-type inhibitors derived from snake venom have been reported to affect blood coagulation and fibrinolysis, key processes in tumor metastasis and angiogenesis. Since they affect platelet function and blood coagulation, these inhibitors could serve as the basis for new cancer therapeutics (Morjen et al., 2022; Sierko and Wojtukiewicz, 2007). Research into protease inhibitors from rare venomous land animals provides valuable insights for the development of new cancer therapies. The diversity of these inhibitors, combined with their unique structures and mechanisms of action, provides a rich source of potential drug candidates. Their ability to regulate critical biological processes associated with cancer, such as coagulation, fibrinolysis, and inflammation, underscores their importance for drug discovery and development. For example, serine protease inhibitors from scorpion venom, such as MeKTT-1 from *M. eupeus*, enhance the biological activities of venom components and can therapeutically influence cancer-related metabolic pathways. These inhibitors serve not only as defense mechanisms for scorpions, but also as models for the development of specific protease inhibitors for cancer progression (Ma et al., 2016). Similarly, non-Kunitz serine protease inhibitors such as LmAPI from *L. mucronatus* show potent inhibitory activity against enzymes such as chymotrypsin, expanding the range of venom-derived molecules with potential anticancer properties (Liu et al., 2015). The study of protease inhibitors from venomous terrestrial animals not only deepens our understanding of venom biology but also paves the way for the discovery of new anticancer therapeutics. Their unique properties, including specificity, stability, and diverse biological functions, make them promising candidates for anticancer drug development.

5. Comparative analysis of protease inhibitors from toxic terrestrial and marine animals

In a comprehensive comparative analysis of protease inhibitors from toxic terrestrial and marine animals, we explore the nuanced differences in efficacy, specificity, and their practical application in drug development, as well as the myriad challenges and benefits they bring. Marine organisms, such as cone snails, are known to produce a variety of peptide toxins, including conotoxins, with high specificity for ion channels and receptors such as voltage-gated sodium channels (Nav) and nicotinic acetylcholine receptors (nAChRs). An outstanding example is the ω-conotoxin MVIIA (ziconotide) from the venom of *Conus magus*, which has been approved by the FDA for the treatment of severe chronic pain (Mir et al., 2016). This toxin uniquely targets N-type calcium channels in nerve cells and offers a nonaddictive alternative to conventional pain pills by blocking pain signaling (Pringos et al., 2011). Jellyfish toxins also offer interesting prospects, particularly peptides from jellyfish species such as the box jellyfish (*Chironex fleckeri*), which affect the ion channels of the heart and could contribute to the development of new therapies for heart disease.

On land, venoms from snakes such as the Brazilian pit viper (*Bothrops jararaca*) have driven the development of ACE inhibitors, which are now a cornerstone in the treatment of hypertension and heart failure. The discovery of captopril, one of the first ACE inhibitors, was based on a peptide from the venom of this snake, demonstrating the direct application of peptides from the venom in pharmaceutical development (Alberto-Silva et al., 2015). Scorpion venom, particularly peptides from the Israeli yellow scorpion (*L. quinquestriatus*), also targets a wide range of ion channels with remarkable specificity. For example, chlorotoxin has been shown to target chloride channels in glioma cells, opening up possibilities for the treatment of brain tumors. Spider venoms, which are rich in peptides that affect ion channels and neurotransmitter receptors, are another example of the diversity of terrestrial venoms. Peptides such as ω-agatoxin IV from the funnel-web spider (*Agelenopsis aperta*) inhibit P-type calcium channels and have become valuable tools in neuropharmacological research (Xia et al., 2023).

These examples illustrate the extraordinary specificity and diverse pharmacological potential of venom-derived peptides. Marine venoms, with their broad spectrum of activity, offer unique opportunities for the development of novel therapeutics, while terrestrial venom peptides, although potentially less diverse, provide a rich source of bioactive molecules for a range of health conditions. Both marine and terrestrial venoms are invaluable resources for drug discovery as they provide diverse compounds that could lead to new drugs for the treatment of diseases such as cancer, pain, and neurological disorders (Xia et al., 2023). The exploration of peptides from marine toxins as lead structures for the treatment of chronic pain, inflammation, and cardiovascular diseases in contrast to terrestrial toxins, which are mainly used for the treatment of neurological disorders and pain therapy, demonstrates the broad applicability of these natural substances. However, the complexity of the composition of the toxins, the difficulties in obtaining sufficient quantities for research, and the need for extensive studies emphasize the importance of advanced techniques and sustainable processes in the use of these resources. As research progresses, the potential to discover innovative therapeutics that address unmet medical needs is

both significant and promising, highlighting the role of toxins in drug development and the study of physiological processes and molecular pathways (Peigneur and Tytgat, 2018).

6. The role of venom-based protease inhibitors in cancer research

Venom-based protease inhibitors, derived from a variety of terrestrial and marine venom species, are at the forefront of innovative developments in cancer drug discovery. Their potential for integration into existing cancer therapy systems by targeting pathways and mechanisms critical to cancer progression is increasingly recognized.

Venom-derived protease inhibitors offer a complementary and effective improvement to current cancer treatments such as chemotherapy, targeted therapy, and immunotherapy. Their unique effects and high specificity could potentially create synergies with existing drugs, increasing their efficacy and reducing side effects. For example, the targeted anticoagulant effect of certain snake venoms, which act through selective fibrinogenolysis, could inhibit cancer metastasis and thus address an important challenge in cancer treatment (Op Den Brouw et al., 2021). Targets for new therapeutics and opportunities for drug development: Venom-derived protease inhibitors affect various cancer-related biological processes such as angiogenesis, apoptosis, cell growth, and metastasis. In particular, ICD-85, a venom-derived peptide, has shown promising results in inducing apoptosis in breast cancer cells, indicating its potential as a specific treatment option (Kheirandish Zarandi et al., 2019). The successful use of proteasome inhibitors such as bortezomib in the treatment of multiple myeloma underscores the possibility of targeting the ubiquitin-proteasome system in cancer therapy, a route by which venom-based protease inhibitors could be effective (Cortelazzo et al., 2010).

The integration of venom-based protease inhibitors into existing cancer treatments represents a groundbreaking approach in oncology and could change the landscape of cancer therapy. These natural products, derived from a range of venom species, have unique biochemical properties that enable them to target and disrupt specific cancer-related signaling pathways and mechanisms. Their incorporation into current treatment modalities—chemotherapy, targeted therapy, and immunotherapy—offers a multifaceted improvement in cancer treatment by increasing efficacy, minimizing adverse effects, and overcoming resistance issues. Chemotherapy, a mainstay in cancer treatment, often faces challenges such as systemic toxicity and drug resistance. Venom-based protease inhibitors can be used strategically to selectively target cancer cells, resulting in less damage to healthy cells and potentially fewer side effects. By interfering with specific protease activities that are critical for tumor growth and metastasis, these inhibitors could also sensitize cancer cells to chemotherapeutic agents, improving overall therapeutic success. In addition, targeted therapy aimed at specifically attacking cancer cells based on genetic, protein, or cellular markers could benefit greatly from the inclusion of venom-derived protease inhibitors. Their high specificity for certain receptors or enzymes involved in carcinogenesis could complement targeted therapies and provide a dual mechanism of action that could prevent the development of resistance while targeting multiple aspects of cancer biology. Numerous in vivo and in vitro studies

have demonstrated the efficacy of venom-based protease inhibitors in cancer models. For example, collinein-1 from the venom of *C. durissus collilineatus* has shown an inhibitory effect on potassium channels in the context of cancer, suggesting a novel antitumor mechanism of action (Cortelazzo et al., 2010). However, the transition from preclinical research to clinical trials is a major challenge and requires further investigation to confirm the safety, efficacy, and optimal delivery methods for these agents in human therapy.

Immunotherapy uses the body's own immune system to fight cancer, but not all patients respond to these treatments (Koury et al., 2018). Venom-based protease inhibitors could modulate the tumor microenvironment to improve the efficacy of immunotherapies. By inhibiting proteases involved in evading the immune system, these agents could improve the visibility of cancer cells to the immune system, increase the response to immunotherapies, and expand the group of patients who benefit from these treatments. One of the biggest challenges in cancer treatment is the development of resistance to therapies. Venom-based protease inhibitors offer a new way to overcome this hurdle. Their ability to act on unique and diverse biological pathways could disrupt the ability of cancer cells to adapt and develop resistance, especially when used in combination with existing therapies. This could lead to more durable responses and delay or prevent disease recurrence. The integration of venom-based protease inhibitors into existing cancer therapies requires careful consideration of dosing, timing, and delivery mechanisms to maximize their therapeutic potential while minimizing potential toxicities. Ongoing and future clinical trials are critical to determine the optimal use of these inhibitors in combination with current treatments (Ma et al., 2017).

Advances in delivery systems, such as nanoparticle carriers or conjugation with monoclonal antibodies, could further improve the targeting of venom-derived agents and ensure that they reach the tumor effectively and safely. In addition, personalized medicine approaches, including genomic profiling of tumors, could help identify the patients most likely to benefit from treatment with venom-based protease inhibitors. Toxin-derived protease inhibitors represent a promising avenue in cancer drug development (Moga et al., 2018). Their different modes of action and specificity in targeting cancer biology make them potential candidates for future cancer treatments. While further research and clinical trials are required to fully understand and validate their use in humans, these inhibitors have the potential to be key elements of the next generation of cancer therapies.

7. Challenges and perspectives in the research of protease inhibitors from toxins

The potential of protease inhibitors derived from toxins is increasingly recognized in cancer therapy research. However, this promising field is not without its challenges and requires a forward-looking approach to future research directions and technological innovation. Ethical and environmental concerns are at the forefront, as the extraction of venom from rare and endangered species poses significant ethical dilemmas and potential threats to biodiversity and natural habitats, highlighting the need for ethical practices and environmental protection in venom research (von Reumont et al., 2022). The complicated nature of toxicants leads to technical and analytical hurdles that require complex extraction and identification processes that require advanced analytical methods to overcome these challenges. This is

compounded by logistical obstacles, particularly in obtaining sufficient quantities of venom from rare or endangered species, which require sophisticated techniques for separation, identification, and synthesis, making the research process both resource intensive and technically demanding (Trim et al., 2021). Another critical hurdle is the translation of laboratory results into clinical applications, a process that must ensure the safety, efficacy, and stability of venom-derived compounds in human subjects while taking into account potential immune responses or adverse effects to make these compounds useful for cancer treatment. Addressing these challenges will require concerted efforts in several areas, including ethical sourcing, advanced technological applications, and rigorous clinical trials, to realize the full potential of venom-derived protease inhibitors in the fight against cancer.

Research in the field of venom-derived protease inhibitors for cancer drug development is at a promising point, driven by recent technological advances and interdisciplinary efforts. Modern technologies such as high-throughput screening, proteomics, and bioinformatics are central to accelerating the discovery and characterization of these inhibitors and overcoming the analytical challenges associated with venom research (Mosquim Junior et al., 2022; Jutty Rajan et al., 2017). In addition, synthetic biology and recombinant DNA technologies represent viable alternatives to natural venom extraction that address ethical and environmental concerns by reproducing the activities of venom components without harming wildlife (Lüddecke et al., 2023). Advances in targeted drug delivery promise to improve the therapeutic efficacy of venom-derived protease inhibitors while minimizing potential side effects, representing a significant advance in their clinical application. Venom research is inherently interdisciplinary and requires collaboration between biologists, chemists, pharmacologists, and clinical scientists to foster innovation and accelerate the drug development process (Lüddecke et al., 2023). However, the path to integrating these novel inhibitors into cancer therapy is paved with regulatory and financial hurdles. Addressing regulatory requirements and securing sufficient funding, particularly from government and healthcare organizations, are critical to advancing venom research toward concrete cancer therapies (Tan, 2022). The impact of recent discoveries in the identification of protease inhibitors in venom species has greatly expanded the therapeutic prospects for cancer treatment. Further research and publications in this field are essential to deepen our understanding and realize the full potential of these compounds in oncology. While the path of venom-derived protease inhibitors from the laboratory to the clinic is complex and challenging, the convergence of technological innovation, ethical sourcing, interdisciplinary collaboration, and solid financial support is key to unlocking their potential to revolutionize cancer therapy.

8. Conclusion

In this chapter, the untapped potential of protease inhibitors from rare venomous animals for anticancer drug discovery was thoroughly investigated. A comprehensive study of both marine and terrestrial species has shown that these organisms are rich in bioactive compounds with considerable therapeutic value. The unique properties of these protease inhibitors, such as their specificity and potency, make them interesting candidates for the development of new cancer therapies. The research presented here shows the different ways in which these inhibitors can interfere with cancer development, opening up new

treatment options that could push the boundaries of existing therapies. Nevertheless, this field faces several challenges. Issues of ethics and ecology, the complexity of venom analysis, and the difficulties of clinical application highlight the need for a cautious and forward-looking approach in this area of research. The use of advanced technologies, the promotion of interdisciplinary collaboration, and the application of innovative methods such as synthetic biology are key to overcoming these obstacles and making progress in this field. Future research should continue to focus on the identification and exploration of these compounds and be supported by substantial funding and regulatory support. As knowledge of toxin-derived protease inhibitors expands, their integration into cancer treatment becomes increasingly realistic. These compounds have the potential to significantly improve the development of more precise and effective cancer therapies. Although the path from toxin to viable cancer therapy is complex and fraught with challenges, the potential benefits are significant. Research into protease inhibitors from rare venomous animals represents a pioneering achievement in cancer research and holds the promise of developing ground-breaking and lifesaving therapies.

Declaration of competing interest

The authors declare no conflicts of interest. The authors have read the journal's authorship agreement and policy on disclosure of potential conflicts of interest.

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CHAPTER

20

Molecular warriors: Proteases as sentinels in the war against cancer diagnosis and prognosis

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1. Introduction

Cancer refers to a disease or a condition of abnormal proliferation or growth of the cells, which is caused as a result of adjustments in the gene expression, which leads to dysregulated balance of cell proliferation and cell death. This imbalance leads to the development of a population of cells, which may invade into other tissues and may also metastasize to distant organs or tissues in the body. If left untreated or undiagnosed, it may result in the death of the host organism.

Cancer is a disease, which is seen in multicellular organisms. As mentioned before, this happens due to the abnormal expression of multiple genes, which leads to dysregulation of the normal cell program and functioning and its various activities such as cell division and cell differentiation. This leads to a disproportion in cell replication and apoptosis, which eventually gives rise to the emergence of a tumor. The tumor can be either benign or malignant based upon its characteristics. Malignant tumors differ from benign tumors in various characteristics such as the ability of the tumor to invade into neighboring tissues and metastasize to any or all organs of the body.

From a clinical perspective, cancer comprises a vast array of complex diseases that differ in various aspects such as time of onset, rate of growth, state of cell differentiation etc. It also takes into account factors such as the ease of detection and diagnosis of the cancer, the degree of invasiveness of the tumor and the potential of the tumor to metastasize, the likely course the cancer or the tumor will take, and the degree of the response the cancer will show upon treatment.

Pathophysiological Aspects of Proteases in Cancer
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Many molecular defects may cause the onset of cancer, it may be small- or large-scale alterations, but these mechanisms occur either as indirect or direct effects on the DNA of the host cell. These mechanisms may include abnormal gene transcription, translation or gene mutation, translocation, deletion, and amplification. It finally results in an impairment in the balance of the replication of cells and death of the cells, which eventually leads to the increase in the size of the tumor or cancerous tissue (Trusevych and MacNaughton, 2015).

2. Proteases in cancer

2.1 What are proteases?

Proteases, also known as proteolytic enzymes, refer to a group of enzymes, which play the function of breaking the long chainlike molecules of proteins into small parts or fragments gradually get converted into their base component, which are amino acids. The process occurs by cleavage of the peptide bonds in the proteins by hydrolysis.

Proteases play various roles in the human body such as digestion of dietary proteins to allow absorption of amino acids, blood coagulation, immunological functions, formation of bones, cell apoptosis, and recycling of unused cellular proteins.

In accordance with catalytic classification, proteases are classified into the following seven categories:

1. Serine proteases
2. Cysteine proteases
3. Threonine proteases
4. Aspartic proteases
5. Glutamic proteases
6. Metalloproteases
7. Asparagine peptide lyases

2.2 Proteases as prognostic markers

In normal cells, proteases play various important roles and regulate a diverse set of cellular processes such as gene expression, cell differentiation, and necrosis. But it has also been recently observed that the same proteases play various roles in tumor growth. The neoplastic cells or the tumor cells show their activity by stimulating the proteases. Tumor progression and metastasis depend on the supply of oxygen and nutrients, which are regulated by various proteases in the tumor and its surrounding organs and tissues. It also has been noticed that various tumors increase the levels of various proteases within the cells as shown in (Fig. 20.1) (Ham and Moon, 2013).

Proteases, essential in normal cell functions such as gene expression and cell differentiation, are now recognized for their involvement in tumor growth. Tumor cells exploit proteases to promote their proliferation and invasive behavior, illustrating a dual role for these enzymes in cellular biology.

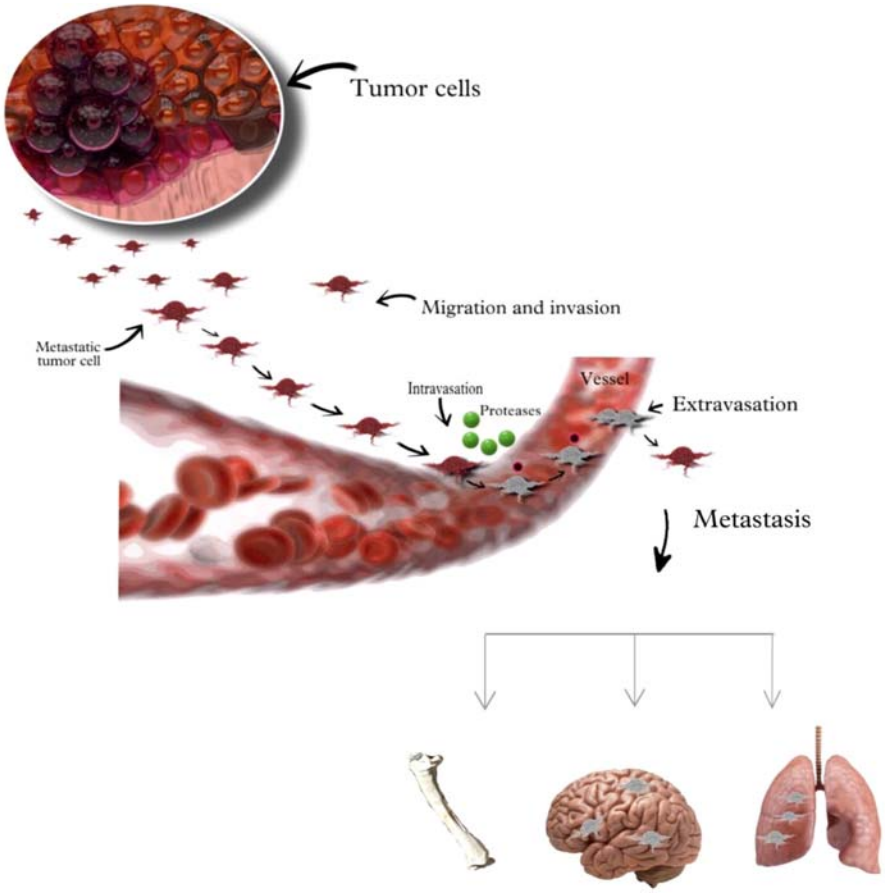

FIGURE 20.1 Migration and invasion of tumor cells. In normal cells, proteases play various important roles and regulate a diverse set of cellular processes such as gene expression, cell differentiation, and necrosis. But it has also been recently observed that the same proteases play various roles in tumor growth. The neoplastic cells or the tumor cells show their activity by stimulating the proteases.

There are various types of proteins that interact with each other to form dynamic networks, which together compose the extracellular matrix. All of these components hold the ability of regulating and influencing the cell differentiation and cell growth. Various studies have now indicated that the extracellular matrix, along with playing various physiological process, also has a significant impact on numerous pathological processes such as metastasis and cancer.

On the basis of various studies that have been conducted, it has been observed that proteases involved in the invasiveness and the metastasis of the neoplastic tissue include urokinase plasminogen activator complex, cathepsins, and matrix metalloproteinases. These proteases that are involved in the degradation of the cellular matrix and its constituents are observed as they are of high clinical use and importance for using these proteases as prognostic markers.

2.2.1 Matrix metalloproteases

Matrix metalloproteases (MMPs) have been associated with the metastasis of tumor cells and its ability of invasion into the neighboring and surrounding tissues as the tumor cells slowly grow and increase in size. This was first observed in studies conducted on animals. In an experiment of transplanting tumor cells in an animal specimen in which the chosen organism was mice, it was observed that the benign tumors that were present in the body of the mice along with higher levels of MMPs in the body were a significant indicator that the benign tumor will acquire the properties of a metastatic tumor gradually, and it also showed in conditions where if the levels of the MMPs were decreasing, then there was also a marked decrease in the invasiveness and the aggression of the spread of the tumor cells. These MMPs have also been observed to induce the proliferation of cells, adhesion, and migration of the cells by the degradation of the extracellular matrix and the release of fragments of proteins in the extracellular matrix and growth factors, but mainly they show their effects in the promotion of invasiveness of the tumor. It does so by specifically opening pathways in the extracellular matrix by the use of which tumor cells could move to distant tissues and lead to the formation of colonies of cancerous tissues in the whole body.

The cancer cells and tissues have also been observed of significantly inducing changes or stimulating the nearby tissues and stromal cells to induce the increased production of the MMPs by interleukins, extracellular MMPs inducers, growth factors, and interferons, which leads to the increase in the invasiveness of the tumor, and it also results in the damage to the basement membranes of the cells.

In a study conducted on the evaluation of the MMPs as a prognostic marker in breast cancer, it was observed that high levels of MMPs show a significant correlation with the recurrence of cancer on a local and regional basis in the patient. Some studies also indicate that there is no use of the proteases as prognostic markers as they were unable to replicate the results in various malignant conditions, whereas some studies showed that higher the levels of the MMPs in the patient's body, the worse the prognosis in the patient.

Finally based upon the studies that were conducted for the evaluation of the MMPs as a prognostic marker, it is understood that for certain cancer cases or certain tumor tissues, there will be the presence of an overexpression of the MMPs, which makes the prognosis of a specific cancer viable, whereas for the other cancers, the same metalloproteases may not act as a prognostic marker due to the variation in the cellular regulators in the cancer or the different pathways, which are active for the progression of the cancer (Ham and Moon, 2013; Heutinck et al., 2010; Lei et al., 2022).

MMPs are inhibited by tissue inhibitors of metalloproteinases, which are also commonly known by the abbreviation as TIMPs. Initially, they were only thought to play an inhibitory role, but their recent studies have proven they are also important for many other activities. One of them under study here will be its use as a viable prognostic marker.

As per a paper written by Elin Hadler-Olsen, Jan-Olof Winberg, and Lars Uhlin-Hansen, both MMPs and TIMPs are capable of being viable prognostic markers in various types of cancer. As mentioned in the paper, MMPs are multifunctional enzymes, which show a variety of effects depending upon the substrate, which is interacting with it along with the biological conditions during that interaction. This translates as a range of effects, which varies between patients, organs, and also in different stages of the development and progression of cancer tissues.

As mentioned, in pulmonary carcinoma, elevated levels of various MMPs such as "MMP-1," "MMP-2," "MMP-7," "MMP-13," and "MMP-26" have been detected in tissue samples obtained from patients diagnosed with lung cancer. Increased levels of MMP-1 have been observed to be excellent markers, which indicate higher risk of development of the metastasis and also are indicative of very low survival rates for patients suffering from lung cancer. In a metaanalytical study conducted on assessment of the relationship between MMP-2 and 1439 "non−small-cell lung cancer" (NSCLC) patients, it was observed from the results that if the tissue samples were showing a strong immunohistochemical staining for MMP-2, it acted as a predictor for low patient survival rates. Similar studies conducted for assessing and establishing a relationship between MMP-9 and NSCLC patients showed that tissue samples staining positive for MMP-9 had lower survival rates as compared with patients whose tissue samples had negative immunohistochemical staining. In some cases, elevated serum concentrations of TIMP-1 and TIMP-2 have been found to be an independent predictor of survival rates of the patients. When TIMP-1 is found in elevated levels, it is an indicator of lower survival rates.

High concentrations of "MMP-1−3," "MMP-7−9," "MMP-11," "MMP-13−15," and "MMP-26" are reported in blood or urine or cancerous tissues in patients diagnosed with breast cancer. Within an exploration conducted on 295 breast cancer patients, high mRNA level of MMP-1 was observed to be associated with unfavorable patient outcomes even though it is not an independent prognostic marker. A strong immunohistochemical staining in the stromal fibroblasts for MMP-1 indicates a high rate of metastatic development. In a research encompassing 1079 mammary carcinoma patients, various polymorphic alternatives of the "MMP-7" gene, which was related to elevated levels of transcription, had an important relation with poor prognosis.

It can be said that MMPs have been observed to be overexpressed in case the tumor or the cancerous tissue is malignant in nature or will develop a metastatic characteristic as it is induced by changes in transcription of genes, which may lead to the presentation of inaccurate prognostic information for a variety of cancers.

On an overall basis, it can be said that MMPs and TIMPs are not yet used as a mainstream prognostic marker due to the conflicting nature of the results of various studies, which are being conducted. But it can be used as a viable option as prognostic markers if all the MMPs are monitored and observed together as a biomarker group (Bushell et al., 2016).

2.2.2 *Urokinase plasminogen activator*

uPA or "urokinase plasminogen activator" is classified under serine proteases that shows multiple actions that gives it the ability to participate various functions such as cellular migration, remodeling of tissues, and the extent of the spread of the cancer. For example, uPA has been observed to be as one of the strongest prognostic markers, and it also acts as a prognostic marker for various subtypes of breast cancer. Some studies have shown that the crucial information regarding breast cancer in patients are obtained by conducting studies on the proteases samples, which have been obtained from the patient, or through the extraction of the proteases from the cancerous tissues, various extraction procedures, for example, proteases, have been used for evaluation of the tissues in the patients diagnosed with breast cancers by acquiring information about it from the extracted proteases, which are extracted by uses of detergents such as Triton X-100. The yield of the extracted proteases is vital to the use of proteases as prognostic markers as different antibodies show different specificity to

different analytical techniques or assay methods and different standards have to be set for different types of uPA category of proteases used for the ELISA tests, which are conducted (Ham and Moon, 2013; Heutinck et al., 2010; Lei et al., 2022).

"Urokinase plasminogen activators" show a major involvement in the prognosis of breast cancers, but they are also observed to show prognostic importance other than only breast cancers. Some studies that are conducted on the basis of a single variable analysis have shown that in gastric cancers, higher levels of the uPA proteases have shown a high significance with increased mortality or decreased levels of expectations of survival for the patients. A similar single variant analysis was conducted on patients suffering from colorectal cancer to study the correlation between levels of uPA and the cancer progression in the patient, but most of the initial data are indicating that the levels of the uPA and cancer progression to the normal mucosa of the gastric mucosal layering is independent of each other. But, by the use of immunochemistry techniques, the staining of the tumor sample tissues was done for uPA, which was found of high prognostic value for cases of certain colorectal cancers such as Duke's B colorectal cancer.

Many preliminary studies have also indicated that uPA also acts as a sign for outcomes for the disease in cancer patients such as bladder cancer, lung cancer, cervical cancer, and ovary, but these proteases are not yet established as a prognostic factor, which acts independently (Heutinck et al., 2010; Karin and Greten, 2005; Keibel et al., 2009).

2.2.3 Cathepsins

Another protease that is involved with malignant conditions is known as "cathepsin." These are a broad category of lysosomal proteases that are of different catalytic types. Within this category of proteases, the proteases that stand out the most are classified as two groups known as aspartic cathepsins, which include cathepsin D and cathepsin E, and the second category that is known as cysteine cathepsins, which include the "cathepsins B, C, F, H, K, L, O, S, V, X, and W." These proteases are mainly stored in lysosomes, but they are able to also be released into the extracellular space, cytosol, or can be released toward the cytoplasmic membrane. These proteases are mainly associated with the initiation of the proteolytic cascades of MMPs and other protease categories such as serine proteases. These cathepsins allow the invasion of the tumor into the surrounding tissues and also lead to playing a role in the establishment of the hallmarks, which can be connected to the malignant forms of cancer via pathways that occur by proteolysis of various proteins and lead to the loss of the functions of those proteins, such as E-cadherin. This allows for the remodeling of the extracellular matrix and causes stimulation of the cellular proliferation and cell apoptosis, and it may also cause the activation of the angiogenesis, promoting tumor invasiveness and development of the metastatic properties of the tumor.

Aspartic cathepsins have been observed to show an effect on the inducing catalytic degradation of the extracellular matrix, and it has also been observed that aspartic cathepsins show an effect in stimulating fibroblast growth and invasion by acting as a paracrine manner.

Similarly, the discovery of cysteine cathepsins has shown that these categories of proteases act as the promoters for the metastatic potential, which is present within the cancer cells. Several other studies of the proteases have indicated that these cysteine cathepsins bring about their effects by two pathways, which might seem to be opposing the pathway for the progression of the cancer, but they have the resultant effect of degradation of extracellular

matrix, which promotes invasion of the tumor in the extracellular space, and it also participates in the cellular apoptotic pathways, which depends on translocating the cathepsins inside the cytosol (Fig. 20.2) (Ham and Moon, 2013; Heutinck et al., 2010; Keibel et al., 2009; Lei et al., 2022; Koblinski et al., 2000).

Various types of proteases, including cysteine, aspartic, threonine, serine, and metalloproteases, are crucial for both cell proliferation and programmed cell death. They facilitate essential cellular processes by regulating protein breakdown and signaling pathways, highlighting their significance in maintaining cell balance.

A study published by "Tao Sun, Daqing Jiang, Liang Zhang, Qinglong Su, Wanli Mao, and Cui Jiang" in Oncology Letters conducted an analysis on the expression of six proteases belonging to the cathepsins family, which includes "cathepsins B, G, D, K, L, and V" in 188 tissue specimens obtained from breast cancer patients by the utilization of immunohistochemical techniques. The levels of the expression of the cathepsins were analyzed in a comparative basis with RECK and expressions of vascular endothelial growth factors.

From the results, it is observed that the expression of all the cathepsins was as follows:

- Cathepsin B—76.76%
- Cathepsin D—58.71%
- Cathepsin G—57.89%
- Cathepsin K—83%
- Cathepsin L—57.23%

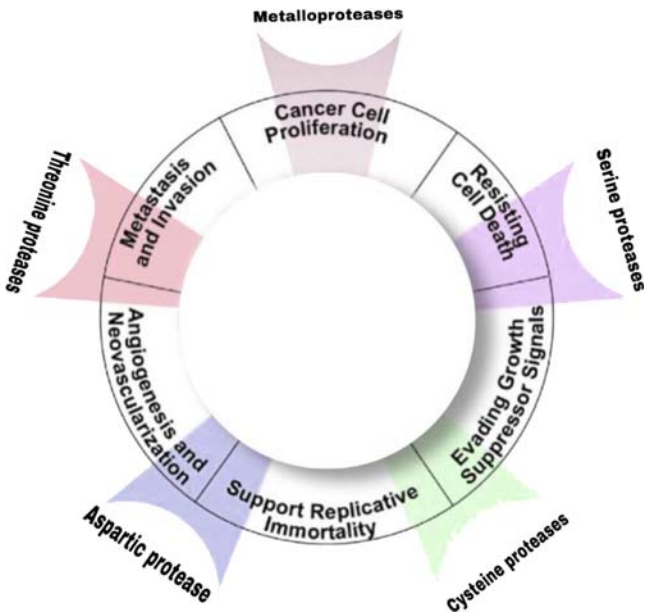

FIGURE 20.2 Different proteases involved in tumor progression. Different proteases such as cysteine protease, aspartic protease, threonine protease, serine protease, and metalloproteases play a key role in cell proliferation and help in cell death.

- Cathepsin V—27.44%
- RECK—38.29%
- VEGF—60.98% (Lara-Ureña et al., 2022; Lee and Choi, 2013)

2.3 Proteases as diagnostic markers

From the experimental studies that have been conducted, it can be said that the set of proteases such as uPA, MMPs, cathepsins, etc. all play a very fundamental role in the progression of cancer and its prognosis. Thus, being said that if it is possible for us to understand the nature of the proteases involved in the cancer progression and it can be used to monitor and evaluate the prognosis of the tumor cells, then it can also be used for the diagnosis of cancer based upon the specific proteases involved in cancer, which are present in the body, which will now act as markers for the presence of cancer and evidence for its presence and its progression in the body (Lee et al., 2004; Leung et al., 2000; Lin and Karin, 2007).

Based upon this, proteases have also been used as a potential diagnostic tool for various experimental studies and are also being developed to be used as specific and viable techniques for the purpose of diagnosing cancer accurately.

There are various main techniques, which are available for the diagnosis of cancer. These techniques include the following:

1. **Tissue biopsy**—This technique allows for the detection of the organ, which is cancerous in nature or either the detection of the affected tissue in any part of the body, which is showing cancerous properties. This confirms the diagnosis of cancer in the affected organ or tissue, which then helps in either the removal of the specific area or part of the organ or either the entire tissue or organ, which is showing cancerous behavior. This test provides with highly reliable results, which can be validated clinically, and it also helps in assessing the tumor and its features directly.
2. **Liquid biopsy**—This technique involves the assay of the collected body fluids or biological fluids from the body, which is then screened for the presence of tumor cells or cancerous tissue. This helps with screening of the cancer, diagnosis of cancer at earlier stages, detection of cellular mutations, which promote cancer conditions. It also helps in monitoring the benefits of the treatment and to evaluate its efficacy and to check for any kind of drug resistance in the treatment being followed. This test is a quick, easy, and noninvasive test, which poses minimum risk toward the patient. It also helps in revealing the heterogenicity of the tumor. However, the results obtained from this test are not very accurate, and it entails the utilization of highly sensitive analytical procedures for the detection of small molecules and the cancerous cells.
3. **Ultrasound scans**—Ultrasound scans are a viable technique for the detection of soft and dense tumor tissues both. It has the ability of locating tumors which are larger than 5 cm in diameter. It is a quick and a noninvasive procedure which has a high sensitivity as it can scan up to depths of 10 cm in the organs, thus allowing better scanning for the presence of cancer, but it is limited by the same fact as it cannot scan any further than its predetermined range due to the scattering of the ultrasound waves, thus leading to inability to scan the cancers, which are rooted deeper in the body.

4. **MRI**—Magnetic resonance imaging or MRI is a computerized radio imaging technique, which is used for the various diagnostic processes for many diseases. In respect to cancer, MRI helps to visualize the tumor, which helps in detection and evaluation of the tumor. It also helps in identifying and detecting the lesions, which are present in the body or tissues surrounding the cancerous tissues or tumor. The benefit of this scanning technique for diagnosing cancer is that there is no use of radiation in this diagnostic process.
5. **CT scan**—This technique determines the extent of the invasion of the tumor in the bone tissues and also helps to scan for the occurrence of metastasis in the cancerous tissues. It gives an easy visual access for understanding the tumor condition and location. These scans also provide high spatial resolution with the use of electron density mapping, thus enabling better visualization of the tumor. The only disadvantages with this technique are it utilizes gamma rays for scanning and it is unable to scan lesions or soft tissues or conduct a staging of the cancer.
6. **PET scan**—This diagnostic test is more suited for assessing the tumor for providing essential information about the biological features and the functional parameters of the tumor under study. It is also very useful in the detection of metastatic lesions as this scan is capable of conducting deep scans and it is highly sensitive in nature.
7. **SPECT scan**—This scanning technique is very useful in detection of bone cancer and metastatic conditions of these cancers. It can also provide images of any inflammation condition if it is present in the body because of the tumor formations. This technique is comparatively cheap to the other scanning techniques and also is able to conduct the test with high sensitivity and can conduct the scan with high penetration depth. But the only limitations for this technique include that is it a very slow scanning process and it does not provide a clear or high-resolution imaging.

With respect to proteases, various diagnostic tests have also been developed for the testing of proteases as a diagnostic marker, but it has been done exclusively for laboratory experimental studies and not as a commercial clinical diagnostic technique for cancer (Liu et al., 2021; Liu and Zeng, 2012; López-Otín and Hunter, 2010). The following techniques are used as scanning of proteases as diagnostic markers for cancer.

2.3.1 Diagnostic techniques using matrix metalloproteases

Optical imaging of MMPs is a technique, which is used for detection and visualization of a variety of biological processes that take place inside a living organism without the use of any kind of invasive probes, but it makes the use of contrast imaging techniques, which use contrast dyes. This technique is based upon the principle of the capability of the cells to induce significant changes in the light, which is acting as a probe. The changes induced mostly are preferred to occur within the visible and near-infrared regions of light. But this method has a requirement, which is that all the fluorescent compounds need to be accumulated at a target site, which will then induce the changes in the light. The first ever probe for the detection of MMPs in in vitro conditions was done by taking an original probe, which senses MMP and then adds a peptide linker to it. Then it was labeled with a specific fluorescent group. This entire sequence can only be cleaved by certain specific MMPs only and will not react or be cleaved by other proteases, which are not specific to this sequence. This

principle was used for conducting diagnosis along with the utilization of SPECT scans for the diagnosis for or imaging of human breast cancer cells, which were transfected with MMP-14, thus achieving the objective of diagnosing the cancerous cells based upon using proteases as diagnostic markers.

Various FRET probes based upon coumarin have been synthesized in which there was a formation of a peptide chain linking between the fluorophore moiety and quencher moiety. This chain was cleaved by specific metalloproteases, which were used in observing and studying the nature by which it was expressing itself when it was done on lines of cervical cancer cells. This fluorescence-based technique was shown to be highly effective and successful in imaging of the MMPs, which are involved in cervical cancer (Lu et al., 2011; Markiewski et al., 2008; Meyer-Hoffert and Schröder, 2011).

On many instances, MRI technique is one of the most extensively used techniques, which is used around hospitals in the entire world. It has been proven to be a highly indicative test for the presence of cancer. This method utilizes the principle of using agents for contrast imaging, which helps in the detection of tumor cells and their evaluation. The agents that are used as traditional contrast agents lack the attachment to a biological ligand, which is mainly responsible for the imaging of the tumors, which is used to study the biological aspects of the tumor, which makes these traditional contrasting agents unsuitable for conducting functional imaging of the tumors. Recent studies have led to the emergence of inventive MRI contrasting components, which are cleaved by the activity of only specific proteases, which allows for the identification of proteases that were overexpressed, which leads to the development of tumors. These are known as protease-modulated contrast agents (PCA), and these help for the assessment of the activity of the in vivo proteases by studying through the imaging done by the MRI (Mignogna et al., 2004; Nielsen and Schmid, 2017).

2.3.2 *Diagnostic techniques using cathepsins*

Quenched fluorescent activity-based imaging techniques are used for imaging of cathepsins as this family of proteases has shown a variety of effects on cancer progression. This technique is performed by the use of activity-based probes, which consists of small molecules that attach themselves to a specific enzyme by chemical interaction. These probes serve the purpose of reading of enzymes by analytical techniques such as fluorescent spectroscopy and mass spectroscopy and also by other biochemical analytical techniques, which are greatly useful in assessment of the overexpression of the specific enzymes, which leads to the development of tumor and its progression (Ohtani, 1998; Olson and Joyce, 2015; Patel, 2017).

Many fluorescent activity-based probes have been developed, which are used for the detection of various proteases such as cathepsins that have been observed to show significant roles to mediate many cell activities and also for the detection of many biomarkers, which are based upon these small molecular targets that are essential for the diagnosis of various diseases. Various studies have established a relationship of the proliferation of the tumor with the expression of the cathepsins involved in it (Hussain and Harris, 2007; Peach et al., 2023; Piotrowski et al., 2020). This technique has led to the development of quenched imaging probes that are proved to be very promising as a clinical tool, which can be used as a noninvasive technique for imaging of cathepsins (Posma et al., 2019; Potempa and Pike, 2009; Prestwich et al., 2008).

Cathepsins can also be observed by imaging via MRI scans. This technique was developed with the aim of conducting imaging that utilizes noninvasive imaging techniques for assessment of in vivo protease activity with regard to imaging cathepsins, which have shown

activity in cancer. This has led to multiple attempts that have been unsuccessful many times, but only one technique has been showing some results that are being used for imaging the cathepsins (Puré, 2009; Quail and Joyce, 2013; Rakash, 2012). This technique is known as near-infrared fluorescence optical imaging or known as NIRF. Although this technique has been showing results with high sensitivity, it lacks the ability to produce 3-D images because of the lack of its deep penetration ability (Raudenská et al., 2023; Ren et al., 2023; Ricklin and Lambris, 2013). This imaging technique was done based upon the proteolysis of MRI probes based upon the activity of specific proteases that show overexpression in tumors (Rochefort and Liaudet-Coopman, 1999; Rothmeier and Ruf, 2012; Sabeh et al., 2009). The function of these imaging techniques is known as chemical exchange saturation transfer (CEST). This technique was seen highly effective in case of brain tumors that were showing high levels of activity of proteases belonging to the cathepsins. It also helps to conduct the assessment of the changes, which occur in vivo by monitoring through CEST contrast imaging, and it also helps in measuring the expression of cathepsin in various types of tumors (Sahai, 2005; Shalapour and Karin, 2015; Shishido et al., 2012).

Another optical technique, which is label free, is known as surface plasmon resonance imaging or known as SPRI. This technique is based upon the changes that are induced upon the light reflectivity when any material is adsorbed on metal surfaces (Shree et al., 2011; Sica et al., 2008).

Two immobilization techniques are used for the detection of the proteases involved in cancer. To quantitatively determine the certain cathepsins, a technique based upon enzyme inhibitor principle is used. The other technique that is used to detect the proteases and its activity is based upon the interaction of antibody−antigen interaction technique (Smith and Kang, 2013; Solinas et al., 2009; Song et al., 2021).

2.3.3 *Diagnostic techniques for urokinase plasminogen activator*

Various optical imaging techniques have also been found out to be very effective in the detection of "urokinase plasminogen activator system." Various studies have reported that ligands are a necessity to conduct optical imaging for uPA receptors, but due to the limited bioavailability of these ligands, the clinical application of this imaging technique is very much limited for diagnostic applications, but this technique can be helpful in tumor removal surgeries where it is guided by image for eradication of the tumor or any kind of malignant tissues from the body (Soualmia and El Amri, 2017; Stuelten et al., 2018).

MRI is also a technique used for imaging the proteases with the help of the ligands (Meyer-Hoffert and Schröder, 2011; Swierczak et al., 2015; van Kempen et al., 2006). This technique allows for higher resolution imaging to get a sufficient number of signals by the MRI scans. The only disadvantage with this is the limited sensitivity that it provides for the scans. To compensate for the lower sensitivity, higher amounts of ligands are needed to be administered for the diagnosis, but it may eventually lead to development of toxicity conditions; thus other noninvasive nuclear scans are preferred such as PET scans and SPECT scans (Vandercappellen et al., 2008; Verhulst et al., 2022; Voss et al., 2004).

SPECT scan has shown a greater potential for clinical applications as it provides a higher sensitivity in scans than the other methods. It also has the ability to find very small micrometastatic lesions and also observe the signs for the detection of invasive cancers (Voss et al., 2004; Wang et al., 2019). It can also be preferred by the basis of the lower cast and the ease of availability of the radiochemistry and the gamma cameras (Wojtukiewicz et al., 2015; Wruck and Adjaye, 2020; Wu et al., 2021).

PET scan can be considered the best imaging technique for detection of uPA receptors as it can produce highly sensitive results, which are combined with accurate quantitative data. But when seen as a whole, PET scans for detection of proteases have been always preferred due to the better results due to the higher sensitivity (Chintamaneni et al., 2022; Dasari et al., 2023; Pindiprolu et al., 2023; Xie et al., 2016).

To study the interaction by advanced research studies between uPA and its receptor through a technique known as surface plasmon resonance and radiography and crystallography has led to developing the chain of small peptides are relevant to conduct the imaging by using PET scans of the uPA receptors (Figs. 20.3 and 20.4) (Keibel et al., 2009; Lee and Choi, 2013; Aminabee et al., 2023; Dasari et al., 2024a,b).

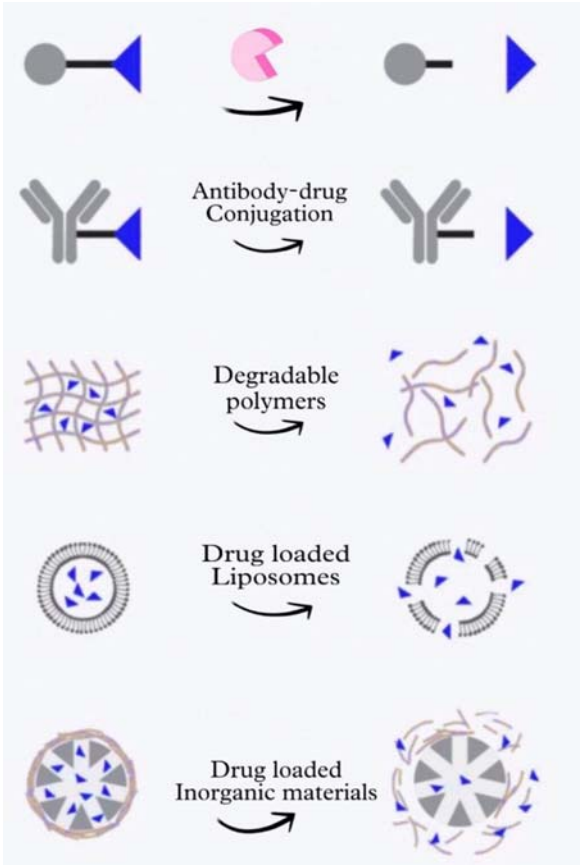

FIGURE 20.3 Therapeutic modalities. Therapeutic modalities for cancer diagnostics and prognosis encompass a diverse range of innovative approaches aimed at improving treatment outcomes. Antibody—drug conjugates (ADCs) combine monoclonal antibodies with potent cytotoxic drugs, targeting specific cancer cells while minimizing damage to healthy tissue. Degradable polymers offer controlled drug release, enhancing drug efficacy and reducing side effects. Drug-loaded liposomes provide a versatile delivery system, encapsulating drugs to target tumors more effectively. Similarly, drug-loaded inorganic materials offer unique properties for drug delivery, improving drug stability and bioavailability. These modalities represent promising avenues in cancer management, offering personalized and targeted approaches to diagnosis and treatment.

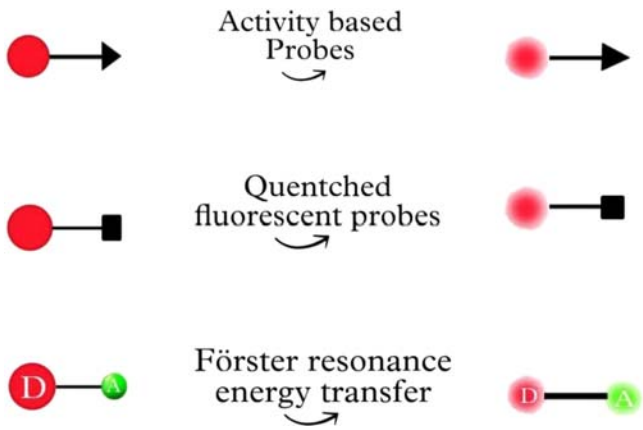

FIGURE 20.4 Diagnostic modalities. Diagnostic modalities in cancer are advancing with innovative techniques such as activity-based probes, quenched fluorescent-based probes, and Förster resonance energy transfer (FRET). Activity-based probes enable the visualization of enzyme activity within tumors, aiding in cancer detection and monitoring treatment response. Quenched fluorescent probes utilize fluorescence to detect specific biomarkers, allowing for precise tumor imaging and early diagnosis. FRET, a sophisticated method, measures molecular interactions within cancer cells, providing insights into disease progression and prognosis. These modalities represent cutting-edge technologies that promise to revolutionize cancer diagnostics, offering earlier detection and more accurate prognostic information for improved patient outcomes.

Therapeutic approaches such as antibody–drug conjugates, degradable polymers, liposomal drug delivery, and inorganic drug carriers are revolutionizing cancer treatment by precisely targeting tumors and minimizing side effects, enhancing patient outcomes. These innovative methods utilize advanced technologies to improve drug delivery and effectiveness, offering hope for more effective cancer diagnostics and prognosis.

New diagnostic methods such as activity-based probes, quenched fluorescent-based probes, and Förster resonance energy transfer (FRET) are enhancing cancer detection by targeting specific biomarkers and visualizing molecular interactions within tumors, potentially leading to earlier diagnosis and improved prognostic accuracy. These advanced techniques offer precise insights into cancer progression, aiding in better patient outcomes through early intervention and tailored treatment strategies.

3. Conclusion

Based upon all the conducted studies, it has now been found out that various proteases are having a significant presence in the tumor and also its adjacent tissues, which acts as a source to meet the elevated oxygen demands and nutritional requirements of the tumor. The proteases show a strong activity or show overexpression in the early stages of tumor formation; thus if suitable methods and techniques are developed for detecting these tumors at earlier stages, this will definitely be a major boost to improve the monitoring of the tumors, which will be able to provide a variety of data, which will be essential for extracting and evaluating the prognosis of the tumor.

The implementation of these nonobtrusive methods is used for the in vivo imaging of these proteases area done by the use of methods such as nuclear strategies, which include MRI, SPECT or known as single-photon emission computed tomography, PET scans or positron emitted tomography, and various optical imaging methods that include fluorescence and bioluminescence.

Probe-based imaging techniques are also viable but have limited use due to the limited availability of the ligands, which are necessary to carry out the imaging of the proteases.

Even though the most used and preferred method for the imaging of the tumors is MRI scans, development and advancement of the other imaging procedures will surely increase the efficiency and options for the accurate diagnosis of the tumors, which are present in the body without the use of invasive techniques, thus allowing the use of proteases as economical, reliable, and safe diagnostic and prognostic markers for cancer.

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SECTION III

Proteases, chemoresistance and cancer

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CHAPTER

21

Serine proteases and their contribution to chemoresistance in cancer

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1. Introduction

The issue of chemoresistance continues to pose a significant obstacle in the cancer treatment, contributing to treatment failure and disease recurrence. Over the past several years, there has been growing interest in the role of serine proteases in contributing to chemoresistance in cancer (Tavano, 2013).

Chemoresistance refers to the ability of cancer cells to become resistant to chemotherapy drugs, which are used to kill cancer cells. Resistance to chemotherapy can manifest through various mechanisms, including enhanced drug efflux, reduced drug absorption, modified drug metabolism, and shifts in the signaling pathways of cancer cells (Yeldag et al., 2018). Chemoresistance can develop during the course of treatment or can be present from the beginning of the cancer diagnosis. It is a major obstacle in cancer treatment and can lead to cancer recurrence and metastasis.

Depending on when it is developed, drug resistance, or chemoresistance, is categorized into two distinct types: intrinsic resistance and acquired resistance. Early detection of various drug resistance helps to maximize efficient treatment, decrease harmful side effects, and predict how sensitive cancer cells will be to a given drug (Ramos et al., 2021).

Intrinsic chemoresistance refers to the inherent or natural resistance of cancer cells to chemotherapy drugs, which can make them less susceptible to the effects of chemotherapy. This can be due to a variety of factors, including genetic mutations, the presence of drug efflux pumps, and alterations in cell signaling pathways.

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Some cancer cells may have mutations in genes that control cell death pathways, making them less responsive to chemotherapy-induced cell death. There are various factors that influence the intrinsic chemoresistance (Lippert et al., 2008). Intrinsic chemoresistance can pose a challenge in cancer treatment, as it can limit the effectiveness of chemotherapy drugs. To address this issue, researchers are investigating new approaches to overcome intrinsic chemoresistance, such as the development of new drugs that can bypass drug efflux pumps or target specific genetic mutations in cancer cells. Additionally, combination therapies that target multiple pathways involved in cancer cell survival and proliferation may be more effective in overcoming intrinsic chemoresistance (Li et al., 2008).

Acquired chemoresistance refers to the development of resistance to chemotherapy drugs by cancer cells after exposure to those drugs. Cancer cells can develop acquired chemoresistance through a variety of mechanisms, including genetic mutations, changes in gene expression, and alterations in cell signaling pathways. One mechanism of acquired chemoresistance involves the upregulation of drug efflux pumps, which can pump chemotherapy drugs out of the cancer cells before they can exert their effects. Another way that cancer cells can survive chemotherapy is by activating DNA damage response pathways. These pathways repair the damage caused by chemotherapy drugs, allowing the cancer cells to continue growing (Aleksakhina et al., 2019; Draghi et al., 2018). Acquired chemoresistance can be a major challenge in cancer treatment, as it can limit the effectiveness of chemotherapy over time and lead to disease progression. To overcome acquired chemoresistance, researchers are investigating new strategies to sensitize cancer cells to chemotherapy drugs, such as the use of combination therapies that target multiple pathways involved in cancer cell survival and proliferation. Additionally, new drugs that can bypass drug efflux pumps or target specific genetic mutations in cancer cells are being developed to overcome acquired chemoresistance.

The causes of this form of resistance encompass drug target mutations, activation of the second proto-oncogene, modifications in the tumor microenvironment, epigenetic alterations induced by methylation, acetylation, and microRNA (miRNA) expression that influence regulators upstream or downstream, adjustments to the cell cycle and its regulatory checkpoints, impaired apoptosis, and changes in DNA repair mechanisms (Wang et al., 2019).

Serine proteases have been demonstrated to have significant implications in cancer, in both tumor growth and progression, and in the metastatic spread of cancer cells to other parts of the body. Serine proteases are also being investigated as potential targets for cancer therapy. Inhibitors of specific serine proteases have been developed as potential treatments for various cancers, and a few of these inhibitors have displayed encouraging outcomes in both preclinical and clinical investigations. For example, inhibitors of uPA have been tested in clinical trials for the treatment of breast cancer and other solid tumors, with some showing promising results (López-Otín and Matrisian, 2007).

Serine proteases have been known for many years, and they are a well-studied class of enzymes. However, there are still many aspects of serine protease biology that are not fully understood, and ongoing research is revealing new insights into the functions and mechanisms of these enzymes. Research on the role of serine proteases in chemoresistance is an emerging area of investigation, and there is growing evidence to suggest that these enzymes may play a role in the development of resistance to chemotherapy drugs in cancer cells.

In cancer, the involvement of serine proteases is intricate and contingent on specific circumstances, necessitating additional research to comprehensively grasp their modes of operation and their potential as candidates for cancer treatment.

In this chapter, we are going to discuss how these terms—proteases, chemoresistance, and cancer are related and what progress has been done in this domain.

2. Overview of serine proteases

2.1 Serine proteases

Serine proteases (or serine endopeptidases) are a type of enzymes that catalyze the hydrolysis of peptide bonds in proteins using a serine residue in their active site. They are selective in their peptide bond cleavage because they exclusively cut the particular protein or molecule to which they are bind. It is the largest family among proteolytic enzymes. This enzyme is found in both eukaryotes and prokaryotes. Almost one-third of all proteases contain a Ser nucleophile in the active site (Cera, 2009). The active site in each serine protease includes a serine reside, a histidine reside, and an aspartate reside, which is distinguished by catalytic triad: Asp−His−Ser (Fig. 21.1). However, this catalytic triad plays a curial in enzymatic activity (Page and Cera, 2008).

Catalytic triad having three amino acids, namely serine, histidine, and aspartic acid is shown in Fig. 21.1.

2.2 Types and classification of serine proteases

Serine proteases are broadly classified into two categories based on their structure such as "trypsin-like" and the "subtilisin -like" of serine proteases. Trypsin-like serine proteases are structurally and functionally similar to trypsin, with a conserved catalytic triad. They are involved in important physiological processes, including digestion, complement activation, blood coagulation, and tissue remodeling. They are also associated with various conditions, such as cancer, inflammation, and autoimmune diseases (Hedstrom, 2002; Gohara and Cera, 2011). Examples of trypsin-like serine proteases include trypsin itself and thrombin. Additional members within the trypsin-like serine protease subfamily comprise chymotrypsin, and elastase, which is tasked with cleaving elastin, a vital constituent of connective tissue.

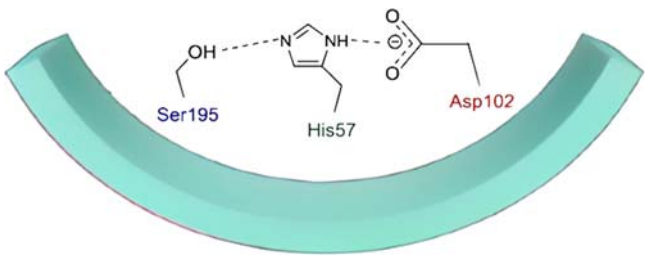

FIGURE 21.1 Catalytic triad of serine proteases. *Created with sketchbook.*

Other members of this subfamily include kallikreins, which are involved in a variety of physiological processes including blood pressure regulation, inflammation, and tissue repair, and are also associated with cancer progression (Carroll et al., 2021).

Subtilisin-like proteases are characterized by both structural and functional resemblances to subtilisin, an enzyme produced by the bacterium *Bacillus subtilis* (Corvey et al., 2003). Like other serine proteases, subtilisin-like proteases have a conserved catalytic triad. Subtilisin-like proteases are found in bacteria, fungi, plants, and animals and play important roles in a variety of physiological processes (Barrett and Rawlings, 1995). Within bacteria, these proteases play a crucial role in overseeing bacterial growth, developmental processes, and the expression of virulence traits. In fungi, subtilisin-like proteases are involved in the breakdown of proteins (Małagocka et al., 2015). In the context of plants, these proteases participate in controlling growth and development, as well as in the plant's defense mechanisms against pathogens (Figueiredo et al., 2017). In animals, subtilisin-like proteases are involved in the digestion of dietary proteins, as well as in the regulation of blood pressure and blood clotting (Patel, 2017).

One example of a subtilisin-like protease in humans is furin, which is involved in the processing of proprotein precursors to their active forms. Furin plays a pivotal role in numerous physiological processes, encompassing aspects of development, immune responses, and the advancement of cancer (Wim et al., 1990).

As these enzymes participate in a broad spectrum of physiological processes, including blood clotting, digestion, and immune response. There are many different types of serine proteases, each with a specific function. Some examples include those mentioned in Table 21.1.

Serine proteases have been the focus of extensive research, and they are important targets for drug development. Inhibitors of specific serine proteases have been developed as potential treatments for various diseases, including cancer, inflammatory disorders, and thrombosis (Clemente et al., 2019).

TABLE 21.1 Serine proteases and its specific function.

Types of serine proteases	Specific function
Trypsin	An enzyme produced in the pancreas that assists in breaking down proteins in the small intestine.
Chymotrypsin	An enzyme produced in the pancreas, which also contributes to the digestion of proteins in the small intestine.
Thrombin	An enzyme involved in the blood clotting cascade that converts fibrinogen to fibrin.
Hepsin	An enzyme that is expressed in the liver and helps regulate blood clotting.
Elastase	An enzyme produced by white blood cells that helps break down elastin, a component of connective tissue.

3. Mechanism of serine proteases in cancer

Serine proteases have been implicated in various aspects of cancer development and progression, including the acquisition of chemoresistance. In cancer cells, the overexpression or dysregulation of serine proteases can lead to changes in cell signaling pathways, alterations in the tumor microenvironment, and resistance to chemotherapy agents. There are several mechanisms by which serine proteases can contribute to chemoresistance in cancer cells.

3.1 Influencing hallmarks of cancer

Serine proteases play a pivotal role in the development and progression of cancer by influencing various hallmarks of cancer. Level of influence relies on various factor such as specific protease, cancer type, stages, and microenvironment. Various serine proteases directly or indirectly contributes in differ hallmarks of cancer, which lead to chemoresistance. Hallmarks of cancer that are impacted by the serine protease are sustaining proliferative signaling, angiogenesis, evading growth suppressors, resisting cell death (apoptosis), avoiding immune destruction, and epithelial-to-mesenchymal cell transitions.

3.1.1 Sustaining proliferative signaling

Matriptase is a serine protease, which acts as both inhibitor and potent tumor-promotor. PAR-2-NF-κB signaling pathway and the PI3K-Akt-mTOR pathway are activated by matriptase and play crucial role in promoting cell proliferation.

Additionally, hepsin, a serine protease, exhibits elevated expression levels in breast and prostate cancer. Hepsin facilitates cell proliferation by activating growth factors such as pro-hepatocyte growth factor (pro-HGF) into its active state, hepatocyte growth factor (HGF). HGF is a strong mitogen and promotes cell proliferation and survival. Hepsin cleaves the extracellular matrix (ECM) protein such as Ln-332, which results in invasion of prostate cancer and elevated cell proliferation. There are several other serine proteases such as Testisin, DESC1, and HALT5 that are associated with cell proliferation, leading to chemoresistance (Martin and List, 2019).

3.1.2 Resisting cell death

The mitochondrial ClpP is a serine protease found in the mitochondrial matrix, forming a stable heptamer structure. Proteolytic activity is due to the formation of multimeric complex with the ATP-dependent unfoldase ClpX (ClpXP). It is essential for the survival of tumor and leads to chemoresistance. Expression of ClpXP is higher than usual in hematological cancers and solid tumor, which leads to chemoresistance. ClpP is overexpressed in various malignancies such as breast, prostate, liver, acute myeloid leukemia, testis, non−small-cell lung cancer, etc. ClpP is crucial for the survival, expansion, resilience, and spread of specific types of cancers. Inhibiting ClpP by utilizing genetic or chemical method causes death of cancer cells with elevated levels of ClpP (Nouri et al., 2020).

3.1.3 Angiogenesis

Folkman proposed a theory stating that the development and spread of solid tumor rely on a process called angiogenesis. Angiogenesis refers to the development and proliferation of fresh blood vessels from preestablished blood vessels in the body (Folkman, 2006). It plays a crucial role in cancer cell proliferation and metastasis by providing a steady supply of nutrients and oxygen (Jiang et al., 2020). There are numerous serine proteases that facilitate the angiogenesis, which leads to the chemoresistance. Elevated levels of mast cells (MCs) have been noted in different types of solid tumors, including gastric cancer, breast cancer, and lung cancer (Qian et al., 2018). Tryptase and chymase, released from mast cell granules, are two serine protease that act as proangiogenic molecules, and contribute to angiogenesis directly or indirectly. Tryptase contributes to upregulating of Ang-1 expression, resulting in the progression in tube formation and growth of endothelial cells. Tryptase has the potential to activate matrix metalloproteinases (MMPs), including MMP-9, and has the ability to convert angiotensin I to angiotensin II. Furthermore, research has indicated that tryptase plays a part in promoting breast cancer angiogenesis by aiding endothelial progenitor cell activation through PAR-2 mediation (Geindreau et al., 2022). The mast cell tryptase (MCT) has been observed to produce a comparable impact to vascular endothelial growth factor (VEGF) in inducing angiogenesis. It has been observed that levels of serum tryptase are considerably higher than usual in pancreatic cancer patients. Elevated levels of MCT were observed to exhibit a positive association with increased microvascular density in individuals diagnosed with pancreatic cancer. These data affirmed that elevated levels of MCT could lead to angiogenesis (Guo et al., 2016).

3.1.4 Epithelial-to-mesenchymal cell transitions

Epithelial-to-mesenchymal cell transition (EMT) is a crucial step in metastasizing cancer that ultimately leads to chemoresistance. EMT is a mechanism through which epithelial cells undergo series of changes to transform into mesenchymal cells, which provides mobility to cancer cells (Fig. 21.2) (Ribatti et al., 2020). Latent EMT-inducing factors are often induced and activated by extracellular proteases. Furthermore, extracellular proteases facilitate the cleavage and subsequent inactivation of cell adhesion molecules. The action of ADAM15 cleaves the E-cadherin. This in turn ensures proficient progression of the EMT process.

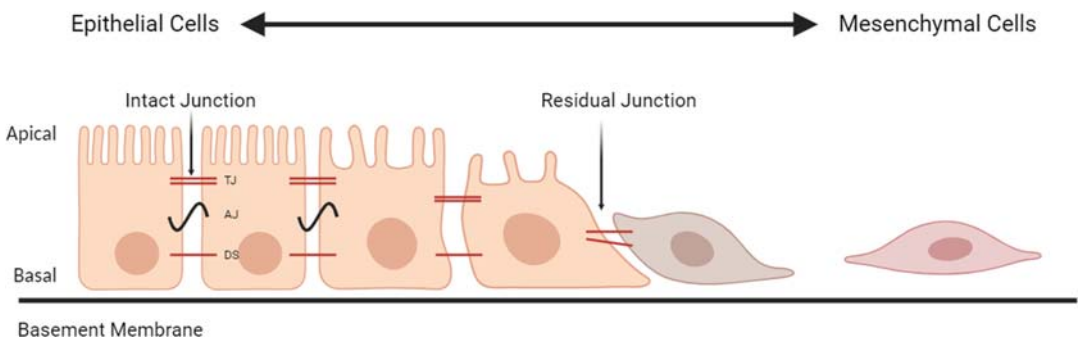

FIGURE 21.2 Epithelial-to-mesenchymal cell transitions. *Created with BioRender.com.*

MMP-14 is a protease enzyme that catalytically increases the accessibility of active TGF-β while leaving total TGF-β levels unaffected. This leads to the induction of EMT in neighboring cells (Mitschke et al., 2019).

Cellular shape-shifting event where tightly packed epithelial cells loosen up and become migratory mesenchymal cells is shown in Fig. 21.2.

Some serine proteases, such as plasmin, can induce EMT, which can promote cancer cell invasion, metastasis, and resistance to chemotherapy (Kalluri and Weinberg, 2010). Plasmin is a serine protease that is involved in the breakdown of ECM components, such as fibrin and laminin. Plasmin can additionally trigger several signaling pathways implicated in tumor advancement and metastasis, which encompasses the stimulation of EMT (Andreasen et al., 2000).

EMT has been shown to promote resistance to chemotherapy and radiation therapy in cancer cells, which can lead to treatment failure and disease progression. Plasmin can induce EMT through a number of mechanisms (Bydoun et al., 2018).

As a whole, the plasmin-induced onset of EMT and chemoresistance represents a conceivable mechanism through which cancer cells can elude chemotherapy and actively participate in the progression of tumors. Targeting plasmin and its downstream signaling pathways may therefore be a promising strategy for improving the efficacy of chemotherapy and preventing tumor recurrence ("Cell Surface Remodeling by Plasmin: A New Function for an Old Enzyme," 2012).

Overall, the role of serine proteases in chemoresistance is complex and multifaceted, and additional research is required to gain a comprehensive understanding of the mechanisms that underlie their involvement in this process. There are other hallmarks of cancer that have a potential link with serine protease and contribute to chemoresistance, but the extent to which these factors exert influence on these hallmarks of cancer remains inconclusive due to limited research in this area.

3.2 Activation of signaling pathways

Some serine proteases, such as protease-activated receptor-2 (PAR-2) agonists, can activate downstream signaling pathways such as PI3K/Akt, which promote cell survival and proliferation (Lei et al., 2022).

PAR-2 is a G protein−coupled receptor that is activated by proteolytic cleavage of its N-terminus by serine proteases, including thrombin, trypsin, and tryptase. PAR-2 activation has been shown to activate the PI3K/Akt signaling pathway in a variety of cell types, including cancer cells (Fig. 21.3) (Heuberger and Schuepbach, 2019). The mechanism by which PAR-2 activates PI3K/Akt signaling involves the recruitment and activation of the scaffolding protein β-arrestin. After PAR-2 activation, β-arrestin binds to the MAPK3, MAPK2, and MAPK receptor and promotes its internalization and trafficking to endosomes. This recruitment of β-arrestin to PAR-2 also leads to the activation of PI3K/Akt pathway signaling (Xu et al., 2015).

Activation of PI3K/Akt pathway through PAR-2 and PI3K/Akt pathway is shown in Fig. 21.3.

One proposed mechanism is that β-arrestin recruits the p85 subunit of PI3K to the receptor complex, leading to PI3K activation. Another proposed mechanism is that β-arrestin recruits

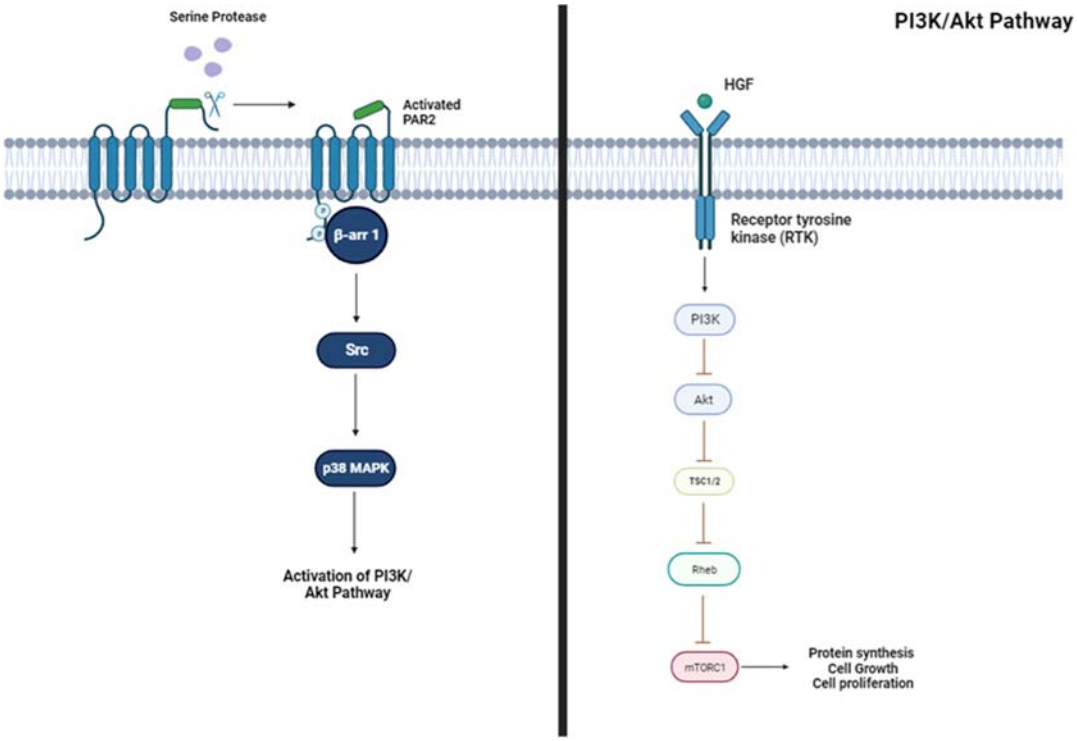

FIGURE 21.3 Activation of PI3K/Akt pathway through PAR-2 and PI3K/Akt pathway. *Created with BioRender.com*

and activates Akt directly, independent of PI3K (Jean-Charles et al., 2017). Once activated, Akt phosphorylates a variety of downstream targets, including ERK2 and GSK3β, which promote cell survival and proliferation. PAR-2 activation of PI3K/Akt signaling can contribute to chemoresistance by promoting cell survival and reducing the efficacy of chemotherapy-induced cell death (Wei et al., 2004).

As a result, focusing on this pathway could serve as a promising therapeutic approach to address chemoresistance in cancer. The PI3K/Akt signaling pathway plays a crucial role in numerous cellular processes, including cell growth, survival, metabolism, and migration. It is activated by a variety of extracellular signals, such as growth factors and hormones, which attach to receptors on the cell surface and initiate a signaling cascade. Activation of these pathways can decrease the sensitivity of cancer cells to chemotherapy-induced cell death (Tsai et al., 2019).

3.3 Remodeling of the tumor microenvironment

Remodeling serine proteases can activate MMPs through a process called proteolytic activation or zymogen activation. MMPs are a group of zinc-dependent endopeptidases responsible for the enzymatic degradation and restructuring of the ECM. They are responsible for breaking down several components of the ECM, such as collagen, elastin, and proteoglycans,

which are critical for maintaining tissue architecture and homeostasis (Wang and Khalil, 2018).

MMPs are synthesized as inactive precursor enzymes, known as pro-MMPs, which require activation by specific proteases to become active (Dreier et al., 2004). The activity of MMPs is tightly governed at the transcriptional, translational, and posttranslational levels, and dysregulation of MMP activity has been implicated in several pathological conditions, including cancer, arthritis, and cardiovascular disease (Fanjul-Fernández et al., 2010).

MMPs can degrade the ECM and promote angiogenesis, allowing tumors to grow and spread. Serine proteases can activate MMPs by cleaving the propeptide domain of the pro-MMP, which exposes the active site and allows the enzyme to degrade ECM components (Quintero-Fabián et al., 2019). Several serine proteases, including urokinase plasminogen activator (uPA), tissue-type plasminogen activator (tPA), plasmin, and kallikreins, have been conclusively shown as contributors to the activation process of MMPs. These serine proteases can activate MMPs directly by cleaving the propeptide domain, or indirectly by activating other proteases that can in turn activate MMPs.

Once activated, MMPs have the capacity to break down a broad spectrum of ECM elements, such as collagen, laminin, and fibronectin. This can lead to the remodeling of the ECM and facilitate cancer cell invasion and metastasis. MMP activation can also contribute to resistance to chemotherapy by facilitating the diffusion of chemotherapeutic agents away from the tumor cells (Bonnans et al., 2014). Therefore, serine proteases play a role in the remodeling of the tumor microenvironment, which can contribute to chemoresistance.

3.4 Interaction with specific chemotherapy agents

Chemotherapeutic agents such as doxorubicin and etoposide require some specific serine proteases for their activation. Alterations in the expression or activity of these proteases can result in resistance to these drugs.

The cleavage of proenzymes, such as MMPs, into their active versions is one potential method by which serine proteases may contribute to doxorubicin activation and chemoresistance. The ECM proteins can then be broken down by activated MMPs, releasing growth factors and cytokines that can activate prosurvival signaling pathways in cancer cells. Doxorubicin's efficacy may be decreased by these signaling pathways upregulating drug efflux pumps such P glycoprotein, which may eliminate the doxorubicin from cancer cells (Liu et al., 2017).

Another possible mechanism involves the activation of procaspases, which are latent enzymes participating in the initiation and execution of apoptosis, a regulated form of cell death. Caspases are a subset of serine proteases, as they also utilize a serine residue for catalytic activity. However, caspases have evolved to serve highly specific functions related to cell death and inflammation regulation. Their activation is tightly controlled and involves proteolytic cleavage at specific sites, resulting in the generation of active subunits that carry out their apoptotic functions (Shi, 2002).

Etoposide is a prodrug that needs to be activated by intracellular enzymes to have an impact on cancer cells. Etoposide is chemotherapy drug frequently used to treat various cancers, such as lung cancer, testicular cancer, and several leukemias. It is a member of the

topoisomerase inhibitors drugs class. DNA topoisomerase II, an enzyme involved in DNA replication and repair, is the main target of etoposide's action. Topoisomerase II is interfered with by etoposide, which can result in DNA damage and cell death (Kluska and Wozniak, 2021).

Cancer cells with enhanced DNA repair systems can more effectively repair the damage brought on by chemotherapy medicines such as etoposide. This lessens the drug's cytotoxic effects. Cancer cells can acquire mutations or alterations in the target molecules of chemotherapy drugs, making them less susceptible to the drug's effects (Wan et al., 2008).

Etoposide interacts with DNA and topoisomerase II during the activation process, which prevents DNA replication and causes DNA damage, cell cycle arrest, and apoptosis. Etoposide's mechanism of action is complex involving multiple steps of DNA cleavage, repair, and cellular responses. Etoposides require activation by specific serine proteases; alterations in the expression or activity of these proteases can lead to resistance to these drugs (Montecucco et al., 2015).

3.5 Modulation of drug efflux pumps

Serine proteases can modulate the expression and activity of drug efflux pumps, such as P-glycoprotein, which can pump chemotherapy agents out of cancer cells and contribute to drug resistance. Serine proteases can modulate the expression and activity of drug efflux pumps through several mechanisms (Król et al., 2010).

One possible mechanism is through the initiation of signaling pathways that upregulate the expression of drug efflux pumps, like P-glycoprotein. Serine proteases can cleave and activate various progrowth factors and cytokines, such as altering growth factor beta (TGF-β), which can activate downstream signaling pathways that lead to the upregulation of drug efflux pumps. This can result in increased efflux of chemotherapy drugs out of the cancer cell, reducing their efficacy (Ughachukwu and Unekwe, 2012).

In addition, serine proteases can directly cleave and activate transcription factors that regulate the expression of drug efflux pumps. For example, studies have demonstrated that serine proteases can trigger the activation of the nuclear factor kappa B (NF-κB) transcription factor, which can upregulate the expression of drug efflux pumps such as P-glycoprotein. Finally, serine proteases can also directly interact with drug efflux pumps to modulate their activity. For example, serine proteases can cleave and activate the extracellular domain of P-glycoprotein, which can enhance its activity and promote drug efflux from cancer (Katayama et al., 2014).

Overall, the modulation of drug efflux pumps by serine proteases represents an important mechanism by which cancer cells can develop resistance to chemotherapy and is an area of active research for the development of novel cancer therapeutics.

4. Cancers associated with over-expressed serine proteases

Serine proteases have been identified for having a significant role in various cancer types, which encompass lung cancer, breast cancer, prostate cancer, ovarian cancer, colorectal cancer, and pancreatic cancer (Table 21.2). For example, studies have shown matriptase and hepsin

TABLE 21.2 Overexpressed serine protease in cancer and their correlation.

Serial number	Associated cancer	Overexpressed serine proteases
1.	Lung cancer	Matriptase, hepsin, uPA, tPA
2.	Breast cancer	uPA, tissue factor pathway inhibitor-2 (TFPI-2)
3.	Prostate cancer	PSA, kallikreins, matriptase, hepsin
4.	Ovarian cancer	FAP-α, hepsin, matriptase, uPA
5.	Colorectal cancer	Matriptase, hepsin, prostasin, MMPs
6.	Pancreatic cancer	Hepsin, matriptase, tryptase, elastase
7.	Melanoma	Matriptase, hepsin, MMPs, uPA
8.	Gastric cancer	Hepsin, matriptase, plasmin
9.	Bladder cancer	Hepsin, matriptase, uPAR
10.	Liver cancer	Hepsin, matriptase, HtrA1
11.	Thyroid cancer	Human HtrA, TMPRSS4
12.	Renal cell carcinoma	Hepsin, uPA
13.	Glioblastoma	MMPs, cathepsin B
14.	Leukemia	uPA, hepsin

have been proven to encourage tumor growth and invasiveness and contribute to chemoresistance by activating PI3K/Akt signaling pathway in lung cancer. In cases of breast cancer, elevated levels of urokinase-type plasminogen activator (uPA), which is a serine protease, have been associated with poor prognosis and decreased sensitivity to chemotherapy. The cleavage and activation of proform of urokinase plasminogen activator (uPA) is carried out by matriptase.

Serine proteases such as prostate-specific antigen (PSA), hepsin, and TMPRSS2 have been demonstrated to facilitate the growth and spread of tumors in prostate cancer. In ovarian cancer, the serine protease FAP-α has been found to be overexpressed in tumor cells and associated with resistance to chemotherapy (Mhawech-Fauceglia et al., 2014).

In colorectal cancer, research has indicated that the serine protease matriptase contributes to the enhancement of tumor growth and contributes to resistance to chemotherapy. It has been observed that levels of matriptase/HAI-1 mRNA are elevated in both colorectal cancer adenomas and carcinomas in contrast to the mRNA from healthy individual tissue.

In pancreatic cancer, the serine protease TMPRSS3 has been observed to exhibit higher expression levels in cancerous cells and associated with poor prognosis and reduced

responsiveness to chemotherapy. The presence of its expression is linked to the metastatic capability of pancreatic cancer cells (Wallrapp et al., 2000). Furthermore, specific prometalloproteases that are strongly expressed in various kinds of cancer cells including pancreatic cancer are activated by plasmin, active form of plasminogen (Johnson et al., 2007).

In gastric cancer, hepsin and matriptase have been observed to have association with tumor growth and invasion.

Matriptase and hepsin overexpression is linked with various cancers such as breast, ovary, uterus, prostate, colon, cervix, and melanoma. Several research studies have shown a downregulation in matriptase mRNA levels in cases of gastric and colorectal carcinomas (Fig. 21.4) (Tanabe and List, 2016).

Flow diagram of serine proteases, their role in cancer, and contribution to chemoresistance are shown in Fig. 21.4.

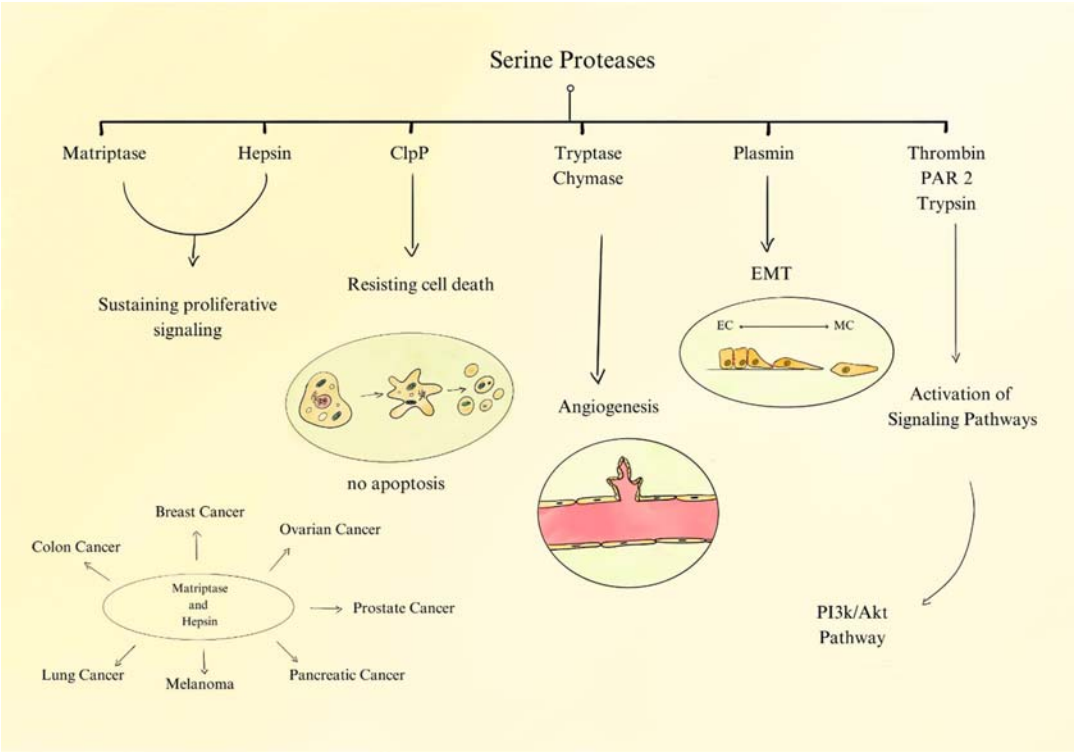

FIGURE 21.4 Flow diagram of serine proteases, their role in cancer, and contribution to chemoresistance. *Created with sketchbook.*

5. Conclusion

Serine proteases have been shown to contribute to chemoresistance in several types of cancer. Serine proteases have been associated with the tumorigenesis, invasion, and metastasis in cancer. Serine proteases can activate cytokines and growth factors that encourage the survival and proliferation of cancer cells. Proteases, such as urokinase-type plasminogen activator (uPA), have been connected to the activation of transforming growth factor-beta (TGF-β), which can increase cell survival pathways and increase chemoresistance. Overexpression and activation of specific serine proteases have been implicated in cancer cell survival, proliferation, and evasion of apoptosis, all of which can reduce the effectiveness of chemotherapy.

Overcoming resistance to chemotherapy continues to be a significant hurdle in cancer treatment. It can arise through a variety of mechanisms, including decreased drug absorption, increased drug efflux, alterations in drug metabolism or activation, activation of prosurvival signaling pathways, and alterations in DNA damage repair mechanisms. Generic mutations, epigenetic modifications, or alterations in the cancer microenvironment may all be responsible for these pathways. Chemoresistance can therefore exist at the time of diagnosis or emerge throughout therapy, and it plays a significant role in both treatment failure and cancer recurrence. For improving the results of cancer treatment, chemoresistance must be overcome. This may involve the creation of novel medications and treatment plans that target certain chemoresistance mechanisms as well as advancements in medication administration and combination therapy.

Despite the difficulties, the emergence of new approaches to treatment and ongoing research into the reasons of chemoresistance offers optimism for raising cancer patient survival rates and quality of life.

6. Future aspect

Serine proteases indeed play a vital role in cancer chemoresistance, and there are several other serine proteases that need to be investigated. As our understanding of the precise functions played by various serine proteases in fostering chemoresistance increases, we may be able to create targeted medicines that limit their activity and make cancer cells more susceptible to chemotherapy. This can involve the development of new drugs and therapeutic strategies that target specific mechanisms of chemoresistance, as well as improvements in drug delivery and combination therapies. The development of innovative drug delivery techniques that specifically target overexpressed serine protease in cancer cells may improve chemotherapy efficiency while reducing side effects.

The survival, proliferation, and evasion of apoptosis of cancer cells have all been linked to overexpression and activation of particular serine proteases, all of which can lessen the efficiency of chemotherapy. Serine proteases can also degrade ECM proteins to aid metastasis and activate signaling pathways such as PI3K/Akt that support chemoresistance.

The creation of novel treatments that can overcome chemoresistance is another area of attention. This may entail the use of advanced drug delivery techniques, such as

nanoparticles and targeted therapies, as well as the development of combination therapies that target a number of resistance pathways.

Overall, the study of serine proteases function in the development of cancer and chemoresistance holds potential for new therapeutic approaches and improved patient outcomes.

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CHAPTER

22

Cathepsins, chemoresistance, and cancer

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1. Introduction

Cathepsins are a group of lysosomal proteases that play important roles in various physiological and pathological processes, including cancer. They are involved in the degradation of extracellular matrix (ECM) components, the processing of growth factors and cytokines, and the regulation of apoptosis. Several studies have shown that cathepsins are upregulated in various types of cancer and are associated with tumor progression and metastasis. In addition, cathepsins have been implicated in the development of chemoresistance, which is a major obstacle to the successful treatment of cancer.

Chemoresistance occurs when cancer cells become resistant to the effects of chemotherapy drugs, leading to treatment failure and disease recurrence. Cathepsins are involved in various mechanisms of chemoresistance, including drug metabolism, drug efflux, and DNA repair. One of the main mechanisms by which cathepsins contribute to chemoresistance is through the regulation of drug efflux pumps. These pumps are membrane transporters that pump chemotherapy drugs out of cancer cells, reducing their effectiveness. Cathepsins have been shown to regulate the expression and activity of drug efflux pumps, leading to decreased drug accumulation and resistance to chemotherapy. In addition, cathepsins are involved in the processing and activation of prodrugs, which are inactive chemotherapy drugs that require enzymatic activation to become effective. By regulating the activation of prodrugs, cathepsins can affect the efficacy of chemotherapy.

Cathepsins also play a role in DNA repair, which is another mechanism of chemoresistance. They are involved in the repair of DNA damage caused by chemotherapy drugs, leading to the survival of cancer cells and the development of resistance.

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Several cathepsin inhibitors have been developed as potential anticancer drugs, which target the activity of cathepsins and reduce their contribution to chemoresistance. However, more research is needed to determine the efficacy and safety of these inhibitors in clinical settings.

In conclusion, cathepsins are important players in cancer progression and chemoresistance. Understanding their role in these processes may lead to the development of more effective cancer therapies and the improvement of patient outcomes.

There are many types of cathepsins, which are classified based on their structure, function, and pH optima. The following are the major types of cathepsins:

1.1 Cathepsin A

Cathepsin A is a serine lysosomal protease involved in intralysosomal proteolysis (Park et al., 2021) It protects galactosidase and neuraminidase-1, and its loss in humans triggers galactosialidosis, a lysosomal storage disorder. Optimal cathepsin A activity occurs at pH 5.6 on carbobenzoxy-L-glutamyl-L-tyrosine, a substrate for pepsin-5. Cathepsin A doesn't need cysteine for activation or iodoacetic acid for inhibition. In the kidney and spleen, a carboxypeptidase activated by SH compounds cleaves carbobenzoxy-L-glutamyl-L-tyrosine. Cathepsins are also implicated in epithelial–mesenchymal transition (EMT) crucial for embryo development. Cathepsin A, a serine carboxypeptidase, is present in the heart's lysosomes and extracellular matrix (ECM). Cathepsin A-mediated degradation of extracellular peptides may contribute to ECM thickening and left ventricle dysfunction.

1.2 Cathepsin B

Cathepsin B is a lysosomal cysteine protease involved in ECM degradation and apoptosis regulation (Turk et al., 2001). It is upregulated in various cancers (breast, lung, prostate, pancreatic), associated with poor prognosis, and implicated in tumor invasion and metastasis. Cathepsin B degrades ECM components such as collagen, fibronectin, and laminin, crucial for tissue structure. This degradation promotes tumor invasion and metastasis. Additionally, cathepsin B regulates apoptosis by influencing caspase activity, impacting cancer cell survival. It also processes antigens for immune cell presentation, affecting the immune response to cancer. Cathepsin B contributes to chemoresistance in cancer by regulating drug efflux pumps and repairing DNA damage caused by chemotherapy drugs. Inhibiting cathepsin B is suggested as a potential cancer treatment strategy, with promising results in preclinical and clinical studies. However, further research is needed to confirm the efficacy and safety of cathepsin B inhibitors in clinical settings. Understanding cathepsin B's role in cancer progression may enhance the development of more effective therapies, improving patient outcomes.

1.3 Cathepsin C

A lysosomal cysteine protease known as cathepsin C (Xiao et al., 2021), often referred to as dipeptidyl peptidase I, is necessary for the catalytic activation of several serine proteases, such as proteinase 3 (PR3), neutrophil elastase (NE), cathepsin G, granzyme A/B/C, and mast cell chymase. Contrary to other cysteine proteases, cathepsin C is a 200-kDa tetramer made up of four identical subunits, as shown by its X-ray crystal structure (Laine and

Busch-Petersen, 2010). Three chains—a heavy chain, a light chain, and an exclusion domain—are included within each subunit. Cathepsin C mutations induce neutrophil serine protease inactivation, which is the root cause of Papillon–Lefevre syndrome. Numerous inflammatory disorders, including Wegener granulomatosis, rheumatoid arthritis, pneumonia, and viral infections, are also linked to cathepsin C.

1.4 Cathepsin D

Cathepsin D is a lysosomal aspartic protease that participates in intracellular protein degradation and autophagy regulation. It exhibits proteolytic activity under low pH conditions in humans. In breast cancer, human epithelial cells overproduce cathepsin D (Cath-D), and its expression levels correlate with a poor prognosis. Cath-D is associated with increased breast cancer cell growth, fibroblast outgrowth, tumor angiogenesis, and metastasis in the breast tumor microenvironment, affecting both cancer and stromal cells. Studies suggest its upregulation in breast cancer and its potential role in the development of chemoresistance (Ashraf et al., 2019).

1.5 Cathepsin E

Cathepsin E is a notable intracellular aspartic protease mainly present in immune system cells, with a significant association with MHC class II pathway-mediated antigen processing (Zaidi and Kalbacher, 2008). Immune cells such as lymphocytes, microglia, dendritic cells, Langerhans cells, interdigitating reticulum cells, and human M cells predominantly express cathepsin E. Recent research has identified that deficiency in cathepsin E leads to a distinct lysosomal storage disease characterized by deposits of lysosomal membrane sialoglycoprotein and an increase in lysosomal pH in macrophages.

1.6 Cathepsin F

Cathepsin F (CTSF) is a newly identified papain-like cysteine protease, playing a crucial role in apoptosis (Yao et al., 2021). In gastric cancer, the expression of CTSF decreases in both tissues and cells. Additionally, the suppression of CTSF significantly reduces the apoptosis of gastric cancer cells.

1.7 Cathepsin G

Cathepsin G is a serine protease playing a role in inflammation and immune response regulation. It is upregulated in different cancer types, potentially contributing to tumor progression and metastasis.

1.8 Cathepsin H

Cathepsin H–activated immune cells produce increased cathepsin H, a lysosomal cysteine protease with distinct aminopeptidase activity. Widely expressed in various organs, such as the lung, pancreas, thymus, kidney, liver, skin, and brain (Ni et al., 2021), cathepsin H plays a

role in regulating the biological characteristics of cancer cells and pathological processes in brain illnesses, owing to its unique enzymatic activity (Fontanesi et al., 2001).

1.9 Cathepsin K

Cathepsin K is a lysosomal cysteine protease responsible for breaking down bone matrix and regulating osteoclast activity. It is upregulated in bone metastases and may contribute to the progression of bone cancers such as osteosarcoma.

1.10 Cathepsin L, S, and O

All belonging to the group of lysosomal cysteine proteases contribute to the degradation of extracellular matrix (ECM) components and exhibit upregulation in various cancer types. This upregulation is associated with tumor invasion and metastasis. Cathepsin L is responsible for processing growth factors and cytokines, cathepsin O is involved in angiogenesis regulation, and cathepsin S participates in antigen processing for presentation to immune cells.

1.11 Cathepsin V

Cathepsin V, a lysosomal enzyme, is found in various tissues such as the cornea, thymus, heart, brain, and skin. It functions as a multifunctional endopeptidase, contributing to MHC class II molecule maturation, antigenic peptide release, breakdown of substrates, elastin fibril turnover, and liberation of antigenic peptides (Lecaille et al., 2022).

1.12 Cathepsin W

Cathepsin W, identified as a cysteine protease, was demonstrated to be indispensable for the replication of influenza A virus through a comprehensive genome-wide RNA interference (RNAi) screen (Edinger et al., 2015).

1.13 Cathepsin X

Cathepsin X, another lysosomal cysteine protease, is predominantly found in cells derived from monocytes/macrophages. In contrast to other human cathepsins, cathepsin X acts primarily as a monocarboxypeptidase, exhibiting rigid positional and limited substrate specificity (Kos et al., 2009).

2. Structure and function of cathepsins

2.1 Structure of cathepsin

One of the first 12 protein crystal structures to be established was papain, a cysteine protease from *Carica papaya* (Fig. 22.1). The structure, together with actinidin, gave researchers the first understanding of their three-dimensional (3D) structure.

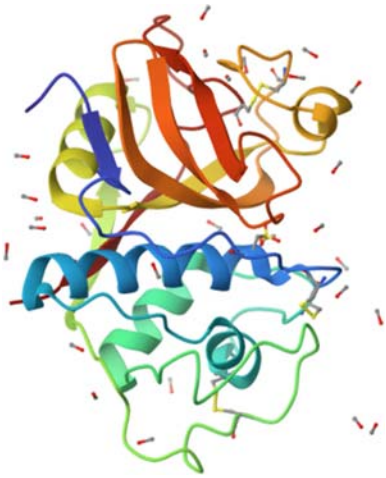

FIGURE 22.1 Structure of papain (PDB DOI: https://doi.org/10.2210/pdb9pap/pdb). A propeptide (19–133), a mature peptide (134–345), and a signal sequence (1–18) make up the 345 amino acid residues that make up the papain precursor protein. The mature peptide serves as the basis for the amino acid numbers. Three disulfide bridges stabilize the protein. Its two separate structural domains and the chasm that separates them make up its three-dimensional structure. The catalytic dyad at the active site, which has been compared to the catalytic triad of chymotrypsin, is located in this aperture. The amino acids histidine-159 and cysteine-25, from which it derives its classification, form the catalytic dyad. It has since been shown that aspartate-158 does not function as well as aspartate in the serine protease catalytic triad.

Papain is a sulfhydryl protease from the latex of the papaya fruit. Its molecules consist of one polypeptide chain with 212 amino acid residues. The chain is folded into two domains with the active site in a groove between the domains. We have refined the crystal structure of papain, in which the sulfhydryl group was oxidized.

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Later discoveries made it possible to isolate cysteine cathepsins from other tissues, including cathepsin B, H, L, S, X, and C. The other cathepsins were expressed in different expression methods. Human cathepsin B's crystal structure was established in the 1990s, and then cathepsins L, K, H, X, V, C, S, and F were established.

The active-site residues (Cys25 and His163, cathepsin L numbering), the residues interacting with the primary chain of the substrate (Gln19, Gly68, Trp183), the N-terminus Pro2, and some Cys residues are all conserved in the mature forms of the nine cysteine cathepsins with a known 3D structure.

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A 3D sequence alignment was performed simultaneously using the crystal structure of human cathepsin L as a template, despite the fact that the 3D structures of the two remaining human cysteine cathepsins, O and W, are still unknown. Cathepsin L, a prominent representative of the cysteine cathepsin endopeptidase, is used in the papain fold. According to the conventional conception, it is divided into two domains, the left (L-) and the right (R-). Three helices make up the L-domain. The vertical helix, sometimes referred to as the core helix is the longest and has more than 30 residues. The front

strand(s) of the R-domain's coiled structure resemble a type of β-barrel. α-helix surrounds the barrel at the bottom. The top of the sheet creating the barrel contains the reactive site histidine. The two-domain interface appears at the top, developing the active-site cleft. The reactive site residues, Cys25 and His163, which originate from various domains, are in its middle (Turkenburg et al., 2002). While histidines were found inside the β-barrel residues of the R-domain, the reactive site cysteine is found at the N-terminus of the central helix of the L-domain. The thiolate-imidazolium ion pair, which is formed by these two catalytic residues, is crucial for the enzymes' ability to catalyze the breakdown of proteins.

The residues from four loops that are present on both domains make up the active-site surface. The short disulfide bonds link the two L-domain loops. The β-barrel hydrophobic core's lid is made up of the two significantly bigger R-domain loops. The substrate binds in an extended configuration along the active-site cleft. Its side chains create alternate interactions with the L- and R-domains. For the side chains of the P3, P1, and P2′ residues, the two loops on the L-domain, and their bridging interface deliver the surface, whereas the loops and residues of the R-domain give the binding surface for the P2 and P1′ residues. P2 residue is an exception to this rule.

The majority of cysteine cathepsins are primarily endopeptidases, whereas cathepsins X and C are exclusively exopeptidases. In addition to being an endopeptidase, cathepsin H and C is also an aminopeptidase. Cathepsin X is a carboxymonopeptidase, whereas cathepsin B is an endopeptidase and a carboxydipeptidase. Exopeptidases have additional structural characteristics that alter the active-site cleft, according to the crystal structures (Musil et al., 1991)

Exopeptidases (cathepsins B, C, H, and X) have additional characteristics that limit the number of binding sites in contrast to endopeptidases (cathepsins F, L, K, S, and V), which have an active-site cleft that runs the whole length of the two-domain interface. In the case of carboxypeptidases, the loops that fill the active-site cleft on the primed sides control substrate binding. Histidine residue(s) are used by these loops in carboxypeptidases, the occluding loop of cathepsin B, and the mini-loop of cathepsin X, to dock the C-terminal carboxylic group of the peptidyl substrate (Fig. 22.2).

Dipeptidyl peptidase I (DPP-I), or cathepsin C (CTSC), is a lysosomal exo-cysteine protease that is a member of the peptidase C1 protein family.

Contrarily, the propeptide regions of cathepsins H and C are used to generate attributes that fill the active-site cleft on the unprimed sides. They dock the positively charged N-terminus of the peptidyl substrates by using the carboxylic groups of the main- and side-chain residues. The carboxylic group in cathepsin H is found at the C-terminus of an eight-residue long propeptide, known as the mini-chain, which is still affixed to the enzyme's body after it has been activated (Gunčar et al., 1998) In contrast, the carboxylic group in cathepsin C originates from the D1 side chain located at the N-terminus of an entire new domain known as the exclusion domain that fills the active-site cleft (Fig. 22.3).

Like other cathepsins, cathepsin L is synthesized as a preproenzyme. A disulfide link connects the heavy and light chains of mature cathepsin L at the C-terminus. At neutral pH, the propeptide inhibits cathepsin L. For catalysis, the triad Cys138, His276, and Asn300 is necessary.

Primary publication DOI: https://doi.org/10.1016/j.bcp.2019.04.006

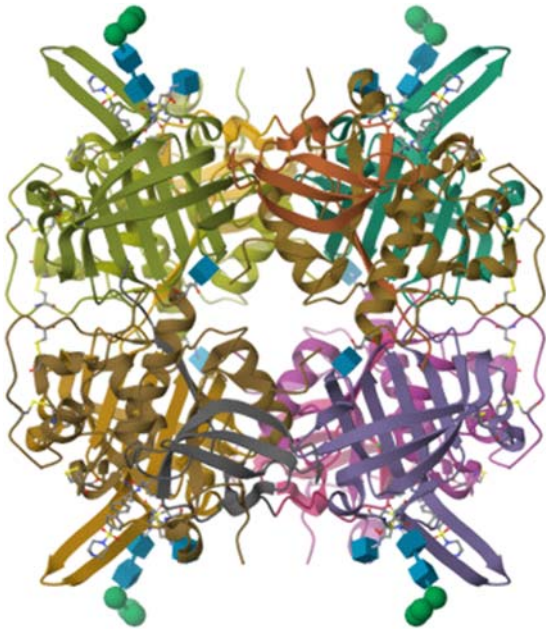

FIGURE 22.2 Structure of cathepsin C (PDB DOI: https://doi.org/10.2210/pdb6IC7/pdb). The polypeptide chain is broken down into at least four pieces to transform cathepsin C into its mature form. A large N-terminal proregion fragment, also referred to as the exclusion domain, is retained in the mature enzyme and is separated from the catalytic domain by excising a minor C-terminal part of the proregion, known as the activation peptide. The signal peptide is removed during translocation or secretion of the proenzyme (procathepsin C). The catalytic domain cleaves to produce a light chain with approximately 69 residues and a heavy chain with approximately 164 residues.

Contrary to the consensus, glycosylation in both aminopeptidases appears to be essential for maintaining the structure of these extra features while also filling the active-site cleft to strengthen substrate binding.

Cathepsin A is a serine protease with an oxyanion hole created by the main-chain nitrogen atoms of Gly57 and Tyr151 and a catalytic triad made up of Asp372, His429, and Ser150 (Schreuder et al., 2014). It is interesting to note that the two hydrogen bonds involving the oxyanion are shorter than typical (2.5Å), probably as a result of the negatively charged oxygen. The ethyl, leucine, and morpholino groups are situated in the active site cleft, whereas the oxadiazole and phenyl groups are situated in the tunnel behind the active site. Multiple van der Waals interactions occur between the leucine residue and Gly57, Pro58, Cys60, Tyr247, Pro301, and Asn339. Its carbonyl oxygen forms a hydrogen bonding with Tyr247's OH, and its nitrogen does the same with Gly57's carbonyl oxygen (Fig. 22.4).

Cathepsin A is a multicatalytic enzyme that exhibits carboxypeptidase activity at acidic pH and deamidase and esterase activities at neutral pH. It is a lysosomal serine protease that shares structural similarities with *Saccharomyces cerevisiae*'s carboxypeptidase Y.

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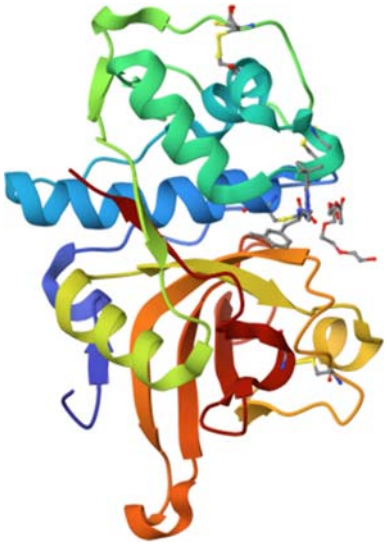

FIGURE 22.3 Structure of cathepsin L (PDB DOI: https://doi.org/10.2210/pdb3hha/pdb). Lysosomal protease cathepsin L (CTSL) is engaged in several cellular processes that have an impact on illness. Like other cathepsins, cathepsin L is synthesized as a preproenzyme. A disulfide link connects the heavy and light chains of mature cathepsin L at the C-terminus.

FIGURE 22.4 Structure of cathepsin A (PDB DOI: https://doi.org/10.2210/pdb6wia/pdb). The lysosomal serine carboxypeptidase known as human cathepsin A (CatA) belongs to the renin–angiotensin system (RAS) and shares structural similarities with acetylcholinesterase (AChE). In addition to deamidinating neurokinin A, CatA is able to extract the C-terminal amino acids of endothelin I, angiotensin I, substance P, oxytocin, and bradykinin.

2.2 Catalytic mechanism and function of cathepsin

All cathepsins belong to one of the following three protease families:

- **Aspartic protease**
- **Serine protease**
- **Cysteine protease**

2.2.1 Aspartic protease

Aspartyl proteases are a subclass of highly specialized proteases that break dipeptide bonds with hydrophobic residues and a beta-methylene group. The cleavage of these proteases does not result in the formation of a covalent intermediate, in contrast to serine or cysteine proteases.

While several other aspartyl protease processes have been put forth, the most commonly recognized one involves the coordination of a water molecule between the two extremely conserved aspartate residues in a generic acid—base mechanism. To undertake a nucleophilic attack on the carbonyl carbon of the substrate scissile bond, one aspartate activates the water by absorption of a proton (Fig. 22.5). This causes the water to produce a tetrahedral oxyanion intermediate that is stabilized by hydrogen bonding with the second aspartic acid. Rearranging this intermediate causes the scissile amide to protonate, which causes the substrate peptide to divide into two product peptides.

One of the four types of proteolytic enzymes that break down other proteins into smaller fragments is aspartic proteases. Because proteolytic enzymes cleave proteins, they are sometimes referred to as proteinases and peptidases, respectively.

2.2.2 Serine protease

The catalytic triad is the key component of the catalytic mechanism of serine proteases. All superfamilies of serine protease enzymes contain the triad, which is situated at the active site where catalysis takes occurs. The three amino acids His 57, Ser 195, and Asp 102 make up the

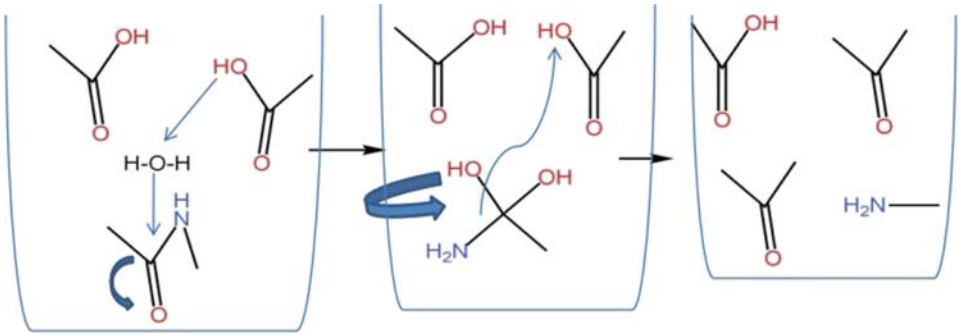

FIGURE 22.5 Reaction mechanism of aspartyl protease. Aspartic proteases are a class of catalytic protease enzymes that catalyze the breakdown of their peptide substrates by binding an active water molecule to one or more aspartate residues. They are also known as "aspartyl proteases" and "aspartic endopeptidases." They are most active at an acidic pH and typically contain two highly conserved aspartates in the active site. Pepstatin inhibits almost all aspartyl proteases that are currently known.

triad, which is a coordinated structure. Each of these three important amino acids plays a key role in the proteases' capacity to cleave.

The mechanism of serine protease can be summarized as follows:

The polypeptide substrate attaches to the surface of the serine protease enzyme, causing the scissile bond to be presented into the active site of the enzyme, with the carbonyl carbon of this bond located near the nucleophilic serine. The nitrogen of the histidine takes in the hydrogen from the [serine] −OH, while the carbonyl carbon is attacked by the serine −OH, and a pair of electrons from the carbonyl oxygen's double bond transfer to the oxygen. Consequently, a tetrahedral intermediate is produced (Fig. 22.6). The peptide bond's link between nitrogen and carbon is now disrupted. The covalent electrons that form this link go to attack the hydrogen in histidine, therefore dissolving the connection. To reestablish the double bond and construct an intermediate acyl-enzyme, the electrons that had previously migrated from the carbonyl oxygen double bond return from the negative oxygen.

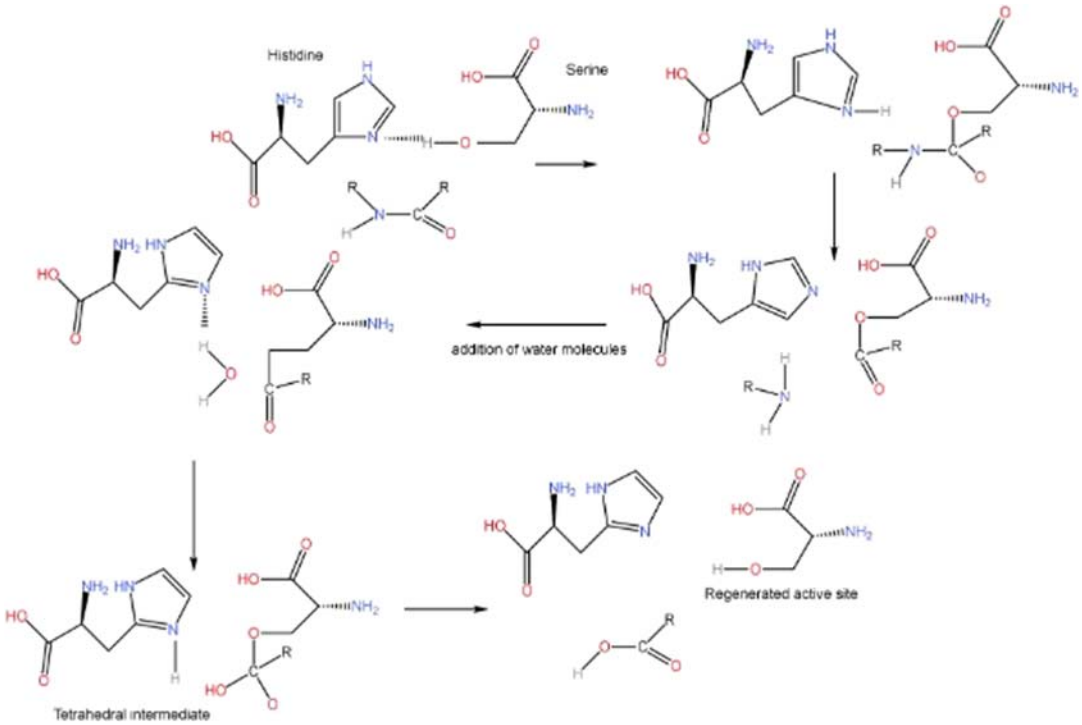

FIGURE 22.6 Reaction mechanism of serine protease. Serine protease is an endopeptidase that breaks down peptide bonds much like any other protease; but, through protein hydrolysis, the serine residues in the active site—which act as nucleophiles—can coordinate a variety of other vital processes. There are several different roles, some of which are crucial, including blood coagulation, digestion, apoptosis, immunity, development regulation, and fertilization. Protease-activated receptors (PARs), G protein−coupled receptors on epithelial, vascular, neuronal, and immunological cells, are the first step in proteolysis, despite their too widespread and complex a role. Its core functions are shared by all living things, including viruses, individuals, and plants.

Protein peptide linkages can be broken by enzymes called serine proteases. At the active site, serine functions as the nucleophilic amino acid. They are present in prokaryotes and eukaryotes alike.

The reaction now includes water. The cleaved peptide's N-terminus is replaced by water, which also assaults the carbonyl carbon. Once more, as the link between the oxygen of the water and the carbon is created, the electrons from the double bond proceed to the oxygen, making it negative. The histidine's nitrogen, which receives a proton from the water, regulates this. Overall, another tetrahedral intermediate is produced.

During the last reaction, the bond that is previously created between the serine and the carbonyl carbon in the first step attacks the hydrogen that the histidine just received. The double bond is reformed with oxygen by the carbonyl atom, which is now electron-deficient. As a result, the peptide's C-terminus is now released.

2.2.3 *Cysteine protease*

The deprotonation of a thiol in the enzyme's active site of the enzyme by a nearby amino acid with a basic side chain, typically a histidine residue, is the initial step in the reaction mechanism by which cysteine proteases catalyze the hydrolysis of peptide bonds (Roy et al., 2022) The anionic sulfur of the deprotonated cysteine then engages in nucleophilic assault on the carbonyl carbon substrate (Fig. 22.7). In this phase, a substrate fragment with an amine terminus is released, the protease's histidine residue is returned to its deprotonated state, and a thioester intermediate is created that connects the substrate's new carboxy

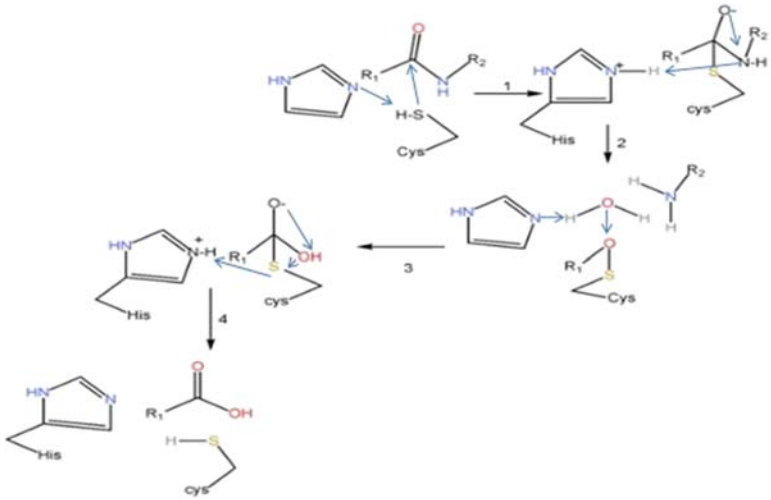

FIGURE 22.7 Reaction mechanism of cysteine protease. One of the four major categories of peptide-bond hydrolases is comprised of cysteine proteases. In peptide-bond hydrolysis, they all employ an S− anion of a cysteine side chain as the nucleophile. All living forms have cysteine proteases, which mediate a broad range of physiological and pathological processes. At one extreme of the spectrum, they are involved in the bulk digestion of protein, while at the other, they are highly regulated rate-determining and specialized peptide-bond cleavages. They are linked to several human diseases, and there is a lot of activity being done in the area of developing cysteine protease inhibitors as medications.

terminus to the cysteine thiol. As a result, they can also be referred to as thiol proteases. To generate a carboxylic acid moiety on the remaining substrate fragment and regenerate the free enzyme, the thioester link is then hydrolyzed.

Protein-degrading hydrolase enzymes are called cysteine proteases, or thiol proteases. A nucleophilic cysteine thiol in a catalytic triad is involved in the catalytic mechanism that these proteases share.

3. Cellular and physiological role of cathepsin

Cathepsins perform a variety of proteolytic processes in the compartments of the endocytic pathway, which helps the regular metabolism of the cell and protein turnover. Cathepsins have major functions in the endosomes of immune cells. Cathepsins are recognized for their role in both innate and adaptive immune responses. Research has demonstrated that lysosomal cathepsins can cleave the ectodomains of TLRs 7 and 9, which are found on the membranes of endo/lysosomes and are responsible for recognizing the nucleic acids of microorganisms that have been phagocytosed, particularly during innate immune responses. TLR 7 and 9 activate TLR signaling pathways by recruiting the adaptor protein MYD88 (Creasy and McCoy, 2011).

In the context of adaptive immune responses, T lymphocytes discern processed antigens displayed on the surface of antigen-presenting cells (APCs) through their association with major histocompatibility complex (MHC) molecules. MHC class II molecules showcase peptides sourced from the endo/lysosomal compartment, while MHC class I molecules exhibit processed antigenic peptides originating from the cytoplasm.

Cathepsins take part in autophagy, a crucial catabolic process that transports components of the cytosol to lysosomes where they are later degraded to preserve cell homeostasis (Ward et al., 2016). Cathepsins play a role in regulating growth and development through the metabolism of hormones and growth factors. One example is cathepsin B, which is responsible for breaking down various substrates including glucagon, pituitary hormone, thyroglobulin, and insulin-like growth factor-1 (IGF-I) (Authier et al., 2005).

Cytosolic cathepsins play a crucial role in apoptosis by triggering the activation of other apoptotic proteases. Specifically, cytosolic cathepsins B, D, and L participate in the degradation of Bid, leading to its activation and subsequent migration to the mitochondria. This relocation ultimately leads to the release of cytochrome C from the mitochondria, thereby activating caspases and commencing the apoptotic cell death pathway. The simultaneous degradation of anti-apoptotic proteins such as Bcl-2, Bcl-xL, Mcl-1, and XIAP (X-linked inhibitor of apoptosis) by cathepsins actively supports and enhances the process of apoptosis (Droga-Mazovec et al., 2008).

Furthermore, cathepsin-D-mediated apoptosis in T cells requires Bax activation and the production of apoptosis-inducing factor (AIF) and cystatin c. This procedure, which has been proven independent of Bid cleavage, triggers apoptosis by directly activating the initiator caspase-8. Recent research has demonstrated that cathepsins control necroptosis. Receptor-interacting serine/threonine kinase 1 (Rip1) is necessary for the kinase activity of necroptosis, which is triggered by numerous triggers. According to studies, cathepsins B and S in macrophages are known to cleave the receptor-interacting protein 1 (Rip1) kinase and so prevent excessive necroptosis (McComb et al., 2014).

Through the activation of inflammasomes, cytosolic cathepsins have a role in triggering inflammatory reactions. The expression of IL-1 was suppressed by gene knockouts and siRNA knockdowns of the cathepsins B, C, S, L, and Z. The fact that cathepsins do not directly cleave IL-1 or caspase-1 suggests that they function prior to inflammasome activation. In addition, through unidentified methods, cathepsins Z and S are known to balance off the activity of cathepsins B, C, and L in LMP-mediated inflammasome activation (Orlowski et al., 2015). In contrast to the neutral pH of extracellular space, cathepsins require an acidic pH for optimal action. However, procathepsins, which are generally stable at neutral pH, are frequently released as cathepsins, which are less active. However, procathepsins, which are generally stable at neutral pH, are often released as cathepsins, which are less active.

4. Regulation of cathepsin activity

4.1 Endogenous inhibitors of cathepsins

Serpins and cystatins are two categories of endogenous cathepsin inhibitors, which are their main regulators and can be divided based on the mechanism of inhibition of cathepsins (Turk et al., 2012). Serpins (serine protease inhibitors) attach to their target proteases in an almost substrate-like manner blocking the active site. Contrarily, exosite-binding inhibitors such as cystatins bind a region near the active site, obstructing substrate access to this site. The best-characterized inhibitors are the cystatins (Csts), which are further divided into four families (Colbert et al., 2011).

4.1.1 Stefins

Stefins are single-chain proteins with around 100 amino acid residues that are synthesized without the need of a signal peptide and are devoid of both disulfide linkages and carbohydrates. Although they can also be found in bodily fluids, they are largely intracellular proteins. The majority of significant eukaryotic subgroups include stefins (Anes et al., 2021). Two of its original members, stefins A and B, were discovered in a variety of animals, including humans. Additionally, the first Trp-containing stefin with a protracted N-terminus has been found to be bovine stefin C. Stefins are a member of the cystatin protein family's I25A subfamily. Cytosol and the nucleus are the principal locations for stefins, which include cystatins A and B (family I).

4.1.2 Cystatins

Cystatins (family II) EM, D, S, SA, and SN are secreted and function as extracellular and/or transcellular proteins. Some type II cystatins, such as CstC and F, are internalized by immune cells or translocated from the secretory system, where they accumulate in endosomal/lysosomal vesicles. Compared with stefins, cytostatins are more broadly dispersed proteins. They are single-chain proteins containing 115 amino acids. In contrast to stefins, cystatins include a signal peptide that secretes extracellular proteins across the cell membrane to the extracellular environment. Cystatin C, salivary cystatins (cystatins S, SA, and SN), cystatin D, cystatin E/M, and cystatin F (leukocystatin) are currently the seven members of this

category of inhibitor that have been found. Except for human cystatin F, which has an extra disulfide bridge that stabilizes the protein's N-terminus, all type 2 cystatins have two highly conserved intramolecular disulfide bridges. The subfamily I25B of the cystatin family I25 contains the human type 2 cystatins.

4.1.3 Kinins

It has been known for some time that kininogens are the building blocks of the kinins, which are mostly present in the blood plasma of mammals and certain other animals. Although one of two primary mechanisms may release kinins using the tissue kallikrein or plasma kallikrein–kinin system, a third pathway may include cysteine cathepsins. The three unique forms of kininogens—high-molecular-weight kininogen (HK), low-molecular-weight kininogen (LK), and T-kininogen (TK), an acute phase protein exclusively detected in rats—are multifunctional and multidomain glycoproteins. The amino acid composition of the heavy chains in HK and LK is the same, while HK's light chain is much longer than LK's. HK has a molecular mass of 84 kDa and LK has 51 kDa. Three tandemly repeated type 2 cystatin-like domains (domains 1, 2, and 3) with eight disulfide bridges make up the heavy chains of HK and LK.

Kininogens, which circulate in the bloodstream as the precursors of the vasoactive peptide kinin, are members of the type III cystatin family. Kininogens are also present in large quantities in sperm, urine, and the lungs.

4.1.4 Thyropins

Thyropins were described as family IV with the discovery that a fragment of the p41 invariant chain (p41Ii) associated with MHC class II molecules inhibits cathepsin L.

4.1.5 Other inhibitors

Some serpins, which are typically serine protein inhibitors, can also block cysteine proteases through a process known as cross-class inhibition. Hurpin selectively inhibits only cathepsin L, whereas human squamous cell carcinoma antigen-1 (SCCA1) is a powerful inhibitor of cathepsins K, L, and S. Comparative to elastase, the serpin endopin 2C specifically inhibits cathepsin L.

4.2 Other regulators of cathepsin

The regulatory function of cathepsins involves transcription, translation, posttranslational, and epigenetic processes (Yadati et al., 2020). Except for cathepsin D, all other cathepsins exhibit TATA-independent transcription initiation at the transcriptional level, defining the initiation site of transcription. For beginning and regulation of transcription, cathepsin L, K, C, and B are known to need a variety of transcription factors, including nuclear factor (NF–Y), specificity proteins, Sp1, Sp3, and erythroblast transformation-specific (Ets) family factors (Jean et al., 2002). Peroxisome proliferator–activated receptor in dendritic cells and estrogen in breast cancer cells are known to control the transcription of cathepsin D (Nakken et al., 2011). Alternative splicing has been observed to cause cathepsin B and L transcript variations. These transcripts have various levels of mRNA stability, and as a result, it is known

that they accumulate during tumors. It has been discovered that the internal ribosome entry site (IRES) controls the translation of cathepsin L. Cysteine cathepsins are epigenetically controlled by methylation because it is known that they also include CpG islands in their promoter region (Mohamed and Sloane, 2006) In addition, endogenous protein inhibitors including stefins, cystatins, and kininogens regulate the activity of the cathepsin enzyme.

4.3 Posttranslational modifications of cathepsins

The catabolization of proteins and the synthesis of new proteins are two crucial functions of cathepsins. Posttranslational processing and modification play a vital part in organelle, a targeting and activity regulation process (Katunuma, 2010). In polysomes, cathepsins are translated in their pre-pro-mature forms. The mature portion of the peptide is targeted into the lysosome via the mannose-6-phosphate signal, and the mature part is bound with their coenzymes. The mature part is removed cotranslationally, the propart is removed, the mature part is glycosylated, and the mature part is targeted into the Golgi apparatus. By limiting proteolysis of the ordered nicked bonds to create hydrophobic peptides, the mature part degradation is initiated. After being ubiquitinated, the peptides are absorbed into phagosomes or proteasomes where they are broken down into amino acids.

4.4 Cathepsins in disease

4.4.1 Role of cathepsin in cancer

Cathepsins are proteolytically active when bound to other cell surface proteins. Cancer cells can penetrate nearby tissues, blood arteries, and lymphatic systems because of their extracellular activity, which also enables them to spread to other locations. Every cathepsin is created from inactive precursors. In the acidic pH of the lysosomes, the endopeptidases are activated by autolysis, and the exopeptidases are activated by the endopeptidases. Some cathepsin family members are highly expressed in cancer are discussed in the following and shown in Table 22.1:

Cathepsin A activity was substantially greater in lysates from malignant melanoma metastatic lesions than in lysates from the original focus. Consequently, cathepsin A may contribute to the metastatic spread of malignant melanoma (Kozlowski et al., 2000). Upregulation of cathepsin B mRNA enhanced cathepsin B staining and increased cathepsin B activity, which has been identified in cancer-invasive edges, implying that cathepsin B plays a role in tumor invasion. A B16 melanoma variant with high metastatic potential has dramatically increased cathepsin B activity in the tumor cells. The incidence of hematogenous and intrapulmonary metastases is often greater in lung cancer patients with increased cathepsin B (Fujise et al., 2000). In addition, a substantial increase in cathepsin B activity was seen in tumor-infiltrated regional lymph nodes compared with noninfiltrated nodes. Patients with non−small-cell lung cancer who had higher levels of cathepsin B experienced metastases more frequently than those who had lower levels of the enzyme. This suggests that cathepsin B is crucial for the invasion and dissemination of tumor cells.

Cathepsin B expression in invasive carcinomas was positively linked with tumor invasion depth and lymphatic metastasis in cervical cancer growth and progression. The cathepsin B

TABLE 22.1 Role cathepsin cancer.

Cathepsin	Elevated in cancer	Location of cancer
A	Yes	Malignant melanoma
B	Yes	Breast carcinomas, melanoma, gastric cancer, lung cancer, colon cancer, ovarian cancer, cervical cancer pancreatic carcinomas, glioblastoma thyroid carcinoma, cholangiocarcinomas, hepatocellular carcinomas, bladder caner
C	Unclear	
D	Yes	Thyroid carcinomas, squamous cell carcinoma, renal cell, carcinoma, glioma brain tumors, laryngeal carcinoma, breast cancer, lung cancer, ovarian carcinoma
E	Yes	Pancreatic ductal adenocarcinoma, gastric cancer
F	Yes	Cervical carcinoma
G	Yes	Breast cancer
H	Yes	Breast carcinoma, colorectal cancer, melanoma, head and neck carcinoma, glioma, prostate cancer
L	Yes	Breast cancer, lung cancer, gastric cancer, colon cancer, head and neck carcinomas, melanomas, gliomas, ovarian cancer, pancreatic cancer
K	Yes	Gastric cancer, squamous cell carcinoma, basal cell carcinoma, breast tumor, lung cancer, melanomas, prostate tumors, renal tumor
O	Unclear	
S	Yes	Astrocytoma, gastric cancer, hepatocellular carcinomas, glioblastomas, melanoma, gastric cancer, pancreatic islet cell cancer
V	Unclear	
W	Unclear	
X	Yes	Prostate cancer, gastric cancer, malignant melanomas, prostate cancer, lung tumors, breast cancer, colorectal cancer
Z	Yes	Melanomas, gastric cancer, hepatocellular carcinomas, pancreatic carcinomas

gene is silenced in cervical cancer HeLa cells by siRNA, which also results in a substantial decrease in the proliferation, migration, and invasion of the HeLa cells (Wu et al., 2012). In low-grade astrocytomas to high-grade glioblastomas, cathepsin B expression steadily arose from three- to sixfold, and both the amount of protein and the enzyme activity increased. Therefore, cathepsin B significantly influenced the growth and invasion of human gliomas.

Breast cancer cells that produce cathepsin D appear to have a role in metastasis and local recurrence. The cathepsin D positive group had considerably more neck node metastases than the cathepsin D negative group in laryngeal tumors (Paksoy et al., 2011). Cathepsin D overexpression and high lymphatic microvessel density may encourage cervical lymph node metastases in laryngeal cancer.

Invasive squamous cell carcinomas (SCC) exhibit much more stromal cathepsin K (Yan et al., 2011) than other epidermal tumors. Therefore, through facilitating extracellular matrix disintegration and thereby aiding SCC development and invasion into neighboring tissue and vasculature, cathepsin K may play an essential part in SCC development. Both perivascular epithelioid cell neoplasms and basal cell carcinoma patients showed cathepsin K expression in the cancer cells.

An essential prognostic marker and prospective target for noninvasive treatment is cathepsin S. In cancers, such colorectal carcinoma, gastric cancer, and hepatocellular carcinoma, cathepsin S has been demonstrated to play unique functions in the migration and invasion of cancer cells. The involvement of cathepsin S in pancreatic islet cell cancer was determined using a gene knockout method, which revealed that mutant forms of the protein hindered tumor invasion. The cathepsin X-mediated inactivation of profilin one's tumor-suppressive activity is a prerequisite for the adhesion, motility, and invasiveness of tumor cells.

5. Therapeutic potential of cathepsins

5.1 Inhibitors of cathepsin activity as potential therapeutics in cancer

Cathepsin inhibitors, both small molecules and antibodies, are being investigated for their anticancer properties. Cathepsin inhibitors can potentially enhance the efficacy of existing cancer therapies, such as chemotherapy and radiation. Cathepsins are involved in antigen processing and presentation, influencing immune responses. Modulating cathepsin activity may impact the tumor microenvironment and immune responses against cancer cells. Combining cathepsin inhibitors with other anticancer agents, such as chemotherapy or immunotherapy, are an area of active research (Kos et al., 2014). Synergistic effects may enhance treatment outcomes and reduce the likelihood of drug resistance. Despite promising findings, it is important to note that the therapeutic targeting of cathepsins is still in the early stages of development, and more research is needed to fully understand their complex roles in cancer biology. Additionally, potential side effects and off-target effects of cathepsin inhibitors need to be carefully evaluated. Clinical trials are ongoing to assess the safety and efficacy of cathepsin-targeted therapies in various cancer types.

The primary physiological function of cystatins is to control excessive cysteine proteinase activity, whether in bodily fluids or cells and tissues. Cysteines often serve as "emergency" inhibitors, encircling and neutralizing peptidase activity in the cytoplasm or extracellular fluids of cells. However, regulatory responsibilities have also been suggested. Until now, it is unclear how cystatins interact with the targeted peptidases because cystatins can be found in the cytoplasm and extracellular fluids, whereas cysteine cathepsins are mostly found in lysosomes. Recent research (Langerholc et al., 2005) shows that external type II cystatins can enter endosomal/lysosomal vesicles and be internalized by immune and tumor cells. Cystatin F stands out among cystatins because it is translocated to endosomes and lysosomes complying with its production. A variety of cellular processes, including antigen presentation and, in the case of tumor growth, the processing of ECM proteins, may be

impacted by the vesicular localization of cystatins. It would be thought that increased cystatin expression would reduce tumor-associated proteolytic activity. Higher levels of stefins A and B, as well as cystatin C, in tumor tissues have been linked to a better prognosis for cancer (Kos et al., 2009)

5.2 Cathepsin-targeted drug delivery in cancer

Cathepsin-targeted drug delivery in cancer is an innovative approach that leverages the overexpression and activity of cathepsins in the tumor microenvironment. This strategy aims to enhance the specificity and efficiency of drug delivery to cancer cells while minimizing off-target effects on normal tissues. Cathepsins, particularly cathepsin B, L, and D, are often upregulated in various cancers, making them attractive targets for designing targeted drug delivery systems.

Lysosomal cysteine protease, cathepsin B is overexpressed in the microenvironment of advanced tumors. Drug−polymer conjugates for the delivery of anticancer drugs have been developed using a variety of polymeric systems. Cancer treatment has extensively used polymer-based drug delivery systems with the cathepsin B−sensitive GFLG sequence. In earlier research (Yang et al., 2017), GFLG sequences were added to PHPMA-based drug conjugates' side chains and polymer backbone to increase therapeutic effectiveness and retaining biocompatibility.

Cathepsin-sensitive linkers have been used to conjugate dox to various polymeric topologies. For instance, a G8-PP1-FK-PABC Dox prodrug emerged when Dox was attached to an octa-guanidine-based peptide sequence (Phe-Lys) through 4-amino benzyloxy carbonyl (PABC) as a self-immolating linker. Lysosomal cathepsin B was able to cleave it and cause selective toxicity against HeLa cells without harming healthy cells (Nair et al., 2015).

The desirable group of inorganic materials (such as silica, gold, iron oxide, quantum dots, etc.) has also been thoroughly researched for the delivery of anticancer drugs. In this field, cleavable rotaxanes were immobilized onto mesoporous silica nanoparticles to create a cathepsin B−induced tumor targeted drug delivery system filled with Dox. The nanocarriers demonstrated effective Dox release, targeted intracellular Dox transport into tumors with strong Cathepsin B expression, and cancer cell imaging. Dendrimers, which are completely monodisperse and highly branched 3D macromolecules, have received a lot of interest, particularly as drug carriers (Zhang et al., 2014). For instance, methoxy polyethylene glycol (mPEG) and Dox surface-functionalized peptide dendrimers have been developed. The resultant dendrimer-GFLGDox nanocarrier, which was enzyme-responsive, provided increased accumulation and retention in ovarian tumor cells (SKOV-3), improving the anticancer impact while exhibiting no overt systemic toxicity.

The peptidic substrate of cathepsin B was created using a sorbitol scaffold functionalized with octa-guanidine moieties and coupled to Dox through a GLPG sequence (Maniganda et al., 2014). Due to an electrostatic contact between the guanidine moieties and the exposed negatively charged phospholipids and sulfates at the cell surface, this compound was effectively taken up by the cells. Following that, cathepsin B used selective cleavage to release Dox into lysosomes. On HeLa cells, which are known to produce cathepsin B, more cytotoxicity was seen than with free Dox.

The biocompatibility of protein or peptide-based drug delivery methods presents an intriguing class of materials (Ahn et al., 2014). In contrast to synthetic nanomaterials, proteinticles, which are proteins that can self-assemble into nanoscale particles inside of cells, can be used in many different biomedical applications because of their improved biocompatibility. It has also been reported that these systems provide cathepsin sensitivity, particularly for small interfering RNA (siRNA) delivery, which has demonstrated significant promise in the treatment of a number of malignancies. For instance, human ferritin-based proteinticles were genetically modified to simultaneously display a variety of functional peptides at their surface, such as cationic peptides for self-assembling siRNA, cancer cell-targeting peptides, or cell-penetrating peptide (Lee et al., 2015). They resulted in improved siRNA capture, cancer cell targeting, and improved tumor cell cytoplasm penetration. Cathepsin B finally broke them down, allowing for the effective intracellular release of siRNA into tumor cells. One of the proteinticles' biggest benefits is that, with simple genetic change, multiple types of functional peptides may be uniformly distributed on their surface according to the type of tumor cell, making it a very flexible approach for targeted siRNA administration.

6. Conclusion and future perspectives

Cathepsins are a class of enzymes that serve specific functions both within and outside of cells. Many mechanical features of cathepsin activity need to be examined, even if recent discoveries have greatly advanced our understanding of these activities and established the possibility of targeting. The biomedical community is currently paying a lot of attention to the extracellular function of cathepsins, establishing them as noninvasive diagnostic indicators and pharmaceutical targets in immunological disorders, cancer, osteoarthritis, and metabolic diseases. There are several selective cathepsin inhibitors being developed that have few adverse effects, function effectively in preclinical animal models, and are suitable for clinical testing. Future research should concentrate on better understanding the unique substrates of cathepsins in the physiological and pathological environment, because of the development of novel mass spectrometry technology and systems biology methodologies. Finally, cathepsins' potential for modified medication delivery appears to be greater than ever. Future research is necessary to identify the translational processes that result in the simplified variants of cathepsin.

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CHAPTER

23

Matrix metalloproteinases, chemoresistance and cancer

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1. Introduction

Cancer is a health challenge responsible for about one in six deaths worldwide. Cancer incidence and mortality are rapidly growing globally, and it is an important obstacle to the increase in life expectancy in this century. Treating cancer has always been a highly complex process. Surgery, chemotherapy, radiation therapy, immunotherapy, endocrine therapy, gene therapy, and targeted therapy alone or in combination are the common methods for treating cancer. However, acquired resistance to chemotherapeutic agents is a major obstacle following long-term treatment, leading to treatment failure and tumor progression/relapse. Several mechanisms of chemotherapy resistance have been characterized, and more continue to be uncovered.

1.1 Occurrence of resistance to chemotherapy in cancers

Finding cost-effective and efficient cancer treatment is an important goal for both scientists and clinicians. Among the various treatment options, chemotherapy is considered the most common cancer treatment modality to reduce the burden of cancer. However, the chemotherapy regimen is unsuccessful in nearly 90% of cases due to the development of resistance by tumor cells to the anticancer agent, resulting in an increased cancer invasion, and metastasis. This increases the difficulty of successful tumor treatment (Vaidya et al., 2022).

Based on the timing of resistance development, chemoresistance can be categorized as intrinsic or acquired resistance, although distinguishing between these two mechanisms can be challenging. Intrinsic resistance or innate resistance is used to describe the presence of resistance mechanisms in tumor cells before therapy is initiated. In this mechanism,

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resistance arises due to tumor-related factors. On the contrary, acquired/extrinsic resistance can be generated after the initial response of tumor cells to the therapy. The modification of nonmalignant cells can lead to intercell signaling that enables malignant cells to survive in the drug's presence. Interestingly, different organs appear to harbor different microenvironments of nonmalignant cells, cytokines, signaling molecules, small molecules, and more which can contribute to cancer resistance (Salemme et al., 2023).

Many cellular and molecular mechanisms are involved in developing chemoresistance, which may act independently or in combination to promote multidrug resistance (MDR) in cancer. According to our knowledge, some of the main mechanisms of chemoresistance are enhanced efflux of drugs by ABC (ATP-binding cassette transporters) transporter family, genetic mutations and epigenetic alterations, increased DNA repair capacity, evasion of programmed cell death (PCD), cancer stemness, and epithelial-mesenchymal transition (EMT) (Emran et al., 2022) (Fig. 23.1).

1.2 Enhanced efflux of drugs

The most common cancer drug resistance mechanism is drug efflux by transmembrane transporters known as ATP-binding cassette transporters (ABC transporters). ABC proteins

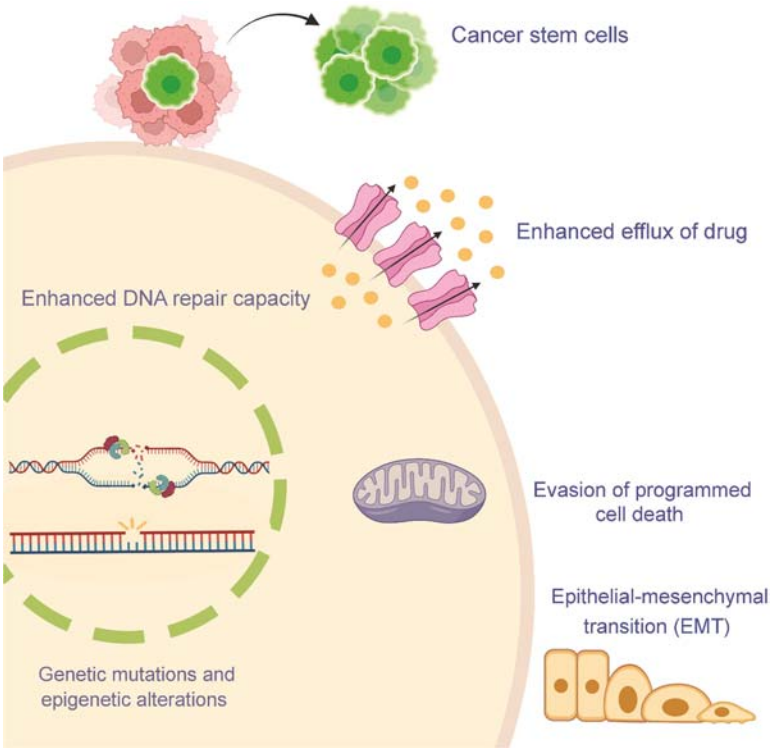

FIGURE 23.1 Chemoresistance mechanisms in cancer cells.

are members of the transport system superfamily and are responsible for the translocation of various substrates across cellular membranes by utilizing ATP hydrolysis (Vaidya et al., 2022). The ABC transporter family consists of 13 distinct members that are directly or indirectly involved in multiple drug resistance development in cancer. The ABC transporter superfamily has been categorized into seven subfamilies based on sequence and structural organization (Alam and Locher, 2023). The most instances of MDR are related to the activity of P-glycoprotein (ABCB1/P-gp/MDR1), multidrug resistance−associated protein-1 (ABCC1/MRP1), and breast cancer resistance protein (ABCG2/BCRP) (Sun et al., 2012).

The expression of ABC transporters is essential for physiological processes (absorption, distribution, and excretion of substrates) in normal tissues, while their overexpression is implicated in MDR in different kinds of hematological and solid tumors such as leukemia, gastric cancer, breast cancer, fibrosarcoma, glioblastoma, hepatic, and lung cancer (Pote and Gacche, 2023). In some cancers, more than one ABC transporter is overexpressed. Therefore, they can confer poor clinical outcomes in the first line of cancer therapy (Xiao et al., 2021).

The expression and function of ABC transporters are affected by intrinsic and extrinsic factors. Previous studies documented that MAPK/ERK and PI3K/Akt pathways are important regulators of the expression and localization of ABC transporter differentially in cancer cells (Crawford et al., 2018). Furthermore, alteration of metabolism in cancer cell lines increases intracellular ATP level, so in chemoresistant cancer cells, ATP level is higher than in chemosensitive cells. According to the ATP-dependent function of ABC transporters, high levels of ATP in cancer cells increase chemodrug efflux (Begicevic and Falasca, 2017). In some cancers, ABC transporters are expressed in high levels naturally, but they are mostly overexpressed after chemotherapy in response to the toxic effects of drugs (Theile and Wizgall, 2021). Therefore, several attempts have been made to overcome this phenomenon by suppressing the activities of these transporters with inhibitors.

Over the decades, three generations of chemosensitizers that reverse MDR have been developed, but they have not achieved satisfactory clinical outcomes due to limitations of their poor pharmacokinetic properties, toxicity, nonselectivity, and low affinity. Therefore, it is worth developing novel ABC transporter inhibitors with improved pharmacological features to overcome these problems (Choi and Yu, 2014).

1.3 Genetic mutations and epigenetic alterations

Mutations and alterations in gene expression patterns, which are common in tumor cells, play an essential role in the progression of cancer and the emergence of drug-resistant tumor cells. Today, the advancement of molecular techniques, including microarray analysis, NGS, and CRISPR-Cas9 screening has revealed the role of many genes in the failure of chemotherapy (Coyle et al., 2017; McLean et al., 2022). For example, the researchers found overexpression of the 10 hub genes including KIT, FOXM1, FGF2, HIST1H4D, ZFPM2, IFIT2, CCNO, MGP, RHOBTB3, CDC7 in platinum-based drug resistance of high-grade serous ovarian cancer (HGSOC) (Wu et al., 2020). Gene mutations can confer drug resistance through various scenarios, such as reduced drug affinity to the target, increased activation of the drug target, conversion of a drug from an antagonist to an agonist, or disruption of specific protein−protein interactions. P53, also known as the guardian of the genome, is

responsible for genome stability and prevents the accumulation of mutations in the genome by regulating cell cycle arrest, DNA damage response, and inducing apoptosis. P53 gene mutations and dysregulation of the p53 pathway are important in the pathogenesis of many human cancers. The loss of function of p53 by missense mutation, as a hallmark of cancer cells, has been reported in various human cancers (Zhu et al., 2020). However, most anticancer drugs induce DNA damage and, subsequently cell death, relaying on p53 function. But, mutant p53 acquires oncogenic functions that promote cancer invasion, metastasis, and resistance to chemotherapy (Zhang et al., 2020).

Epigenetics is a gene expression regulating mechanism without changes in DNA sequence that determines cell phenotype. Epigenetic mechanisms can regulate gene expression by chemically modifying DNA bases and altering the chromosomal structure. Recent advancements have identified different epigenetic mechanisms: DNA methylation, histone modification, nucleosome positioning, and noncoding RNAs. The regulatory regions of the genome including promoter and superenhancers (SEs) are more susceptible to epigenetic modifications (Bure et al., 2022).

Along with gene mutations, epigenetic alterations are crucial factors in the progression of cancer and chemotherapy resistance. During tumorigenesis, the epigenome undergoes various changes such as global hypomethylation of DNA, hypermethylation in specific regions (especially in promoter-associated CpG islands of tumor suppressor genes), global changes in histone modification as well as alterations in miRNAs expression (Ilango et al., 2020). Activation of oncogenes by promotor hypomethylation and suppression of tumor suppressor genes by promoter hypermethylation contributes to invasion, metastasis, and drug resistance of cancer cells. Chemotherapeutic agents can induce epigenetic reprogramming and cause chemoresistant epigenetic state in a tumor, which was initially sensitive to treatment (Pathak et al., 2023).

A nucleosome is a repeating structural unit in chromatin that consists of a 145−147 bps DNA segment wrapped around eight histone proteins. Nucleosome positioning represents a level of epigenetic regulation of gene expression (Espiritu et al., 2021). The studies highlighted the role of nucleosome and histone modifications in carcinogenesis. Genome studies in breast cancer cells have shown that nucleosome positioning is essential for the interaction of progesterone receptors with their binding sites. Various mutations in histones can lead to dysfunctions in cell mechanisms and have been observed in glioblastoma. For instance, the K27M mutation in Histon3 (H3) is a well known mutation in cancer patients that is effective in response to treatment. The effect of the mutagenesis process depends on accessibility to DNA that is regulated by nucleosome positioning and histone modifications (Rakotomalala et al., 2021).

Usually, multiple histone modifications at the posttranslational level are involved in cancer development and drug resistance, including acetylation, methylation, phosphorylation, ubiquitination, and formylation. For example, the acetylation of histones transforms chromatin structure to a low condensed state that results in high transcription of oncogenes. Also, histone deacetylation in tumor suppressor genes enhances the interaction of nucleosomes with chromatin, which results in gene silencing (Yang et al., 2022).

Noncoding RNAs (ncRNAs) are other types of epigenetic modulators that are not translated to protein. The ncRNAs play a key role in the regulation of gene expression and phenotypic diversity. They consist of long-noncoding RNAs, circular RNA, piwi-RNA, miRNA,

and siRNA. The ncRNAs interact with RNA, DNA, or proteins and, subsequently, suppress the function of their targets. In this way, they can regulate various cellular processes (Kaikkonen et al., 2011). The evidence indicated that ncRNAs have a significant role in the pathogenesis of cancer and the development of resistance to chemotherapy treatments in different types of cancer. For example, lncRNAs-mediated epigenetic modification by DNA methylation and histone modification in cancer cells lead to induce drug resistance through EMT progression, apoptosis evasion, altering drug targets, and drug efflux (Ye et al., 2022).

Although to date only two classes of epigenetic drugs have received the US Food and Drug Administration (FDA) approval including DNA methylation inhibitors (iDNMTs) such as 5-azacitidine and 5-aza-20-deoxycytidine (decitabine) and histone deacetylase inhibitors (iHDACs), i.e., belinostat, romidepsin, vorinostat, and panobinostat, several novel targets are currently undergoing late-stage clinical trials and represent promising results in cancer therapy (Ponnusamy et al., 2020). While both iDNMTs and iHDACs are effective alone, their efficacy is enhanced when used in combination treatment for overcoming cancer therapy resistance. For example, in tamoxifen-resistant breast cancer cells, DNMT3A is upregulated, and its inhibition by iDNMTs results in cancer cell rechemosensitization (Man et al., 2022).

1.4 Enhanced DNA repair capacity

DNA repair pathways are activated in response to various DNA-damaging agents that are essential for the maintenance of stability and integrity in the genome. The disruption of DNA repair pathways is a hallmark of cancer, which contributes to the initiation and progression of cancer. Ionizing radiation and chemotherapy are the primary anticancer therapies that cause cell destruction through directly or indirectly damaging DNA (Lin et al., 2022). Aberrant DNA damage response may lead to cancer cells becoming hypersensitive or resistant to genotoxic agents, and thereby modulating the DNA repair pathways can improve tumor response to treatment (Li et al., 2021).

One significant characteristic of numerous chemotherapeutic agents is the induction of lesions and interstrand or intrastrand interaction in DNA to induce apoptosis through excessive damage (Li et al., 2021). To deal with such lesions, cancer cells have developed different mechanisms called DNA damage response (DDR) pathways including base excision repair (BER), nucleotide excision repair (NER), nonhomologous end-joining (NHEJ), mismatch repair (MMR), homologous recombination (HR), and translesion synthesis (TLS), which are involved in the repair of single-strand breaks (SSB), DNA lesions, and double-strand breaks (DSBs) (Chatterjee and Walker, 2017). The impairment of DNA damage response (DDR) pathways in cancer cells leads to increased resistance to chemotherapy; therefore, it would be valuable to evaluate the DNA repair phenotype before treatment in the clinical management of cancer patients. Accordantly, different DDR pathway inhibitors are currently under clinical development or approved for use in the market (Choi and Lee, 2022).

Cisplatin, as a common chemotherapeutic drug in clinical practice, can cause DNA damage in cancer cells and drive the cancer cells toward death. However, the efficacy of cisplatin is reduced, and cancer cells eventually become drug resistant because of the upregulation of genes that are involved in DNA repair mechanisms. In cisplatin resistance cells, DNA lesions

are tolerated through bypass TLS, NHEJ, MMR, and NER pathways activation. Therefore, the combination therapy of cisplatin and DDR inhibitors helped to enhance the cisplatin sensitivity of resistance cells and improve the effectiveness of cisplatin due to their synergistic effect (Rocha et al., 2018).

1.5 Evasion of programmed cell death

The evasion of different programmed cell death (PCD) including apoptosis, autophagy, and necroptosis is considered key hallmark of carcinogenesis and chemoresistance. The vast majority of chemotherapeutic agents act through the activation of PCD, specifically apoptosis, to kill cancer cells. Furthermore, chemotherapy induces cellular stress signaling that contributes to drug-based apoptosis tolerance. Recent evidence suggests that the tumor cells that exhibit chemoresistance reduced sensitivity to drugs due to disruption in apoptosis (Mollaei et al., 2021).

Chemoresistance cancer cells may escape apoptosis through mutation of proapoptotic and antiapoptotic genes, impaired function of pro- and antiapoptotic proteins due to altered post-translational modifications, and defect in caspases function. The caspase activation cascade can be inhibited by the loss of function of the apoptosome, the ubiquitination of cytochrome *c*, and the inhibition of caspase function by IAPs (Dandoti, 2021). For example, an increase in neural apoptosis inhibitory protein (NAIP) expression leads to cisplatin and etoposide resistance in progressive neuroblastoma (NB) cells. NAIP is enabled to bind to procaspase 9 and caspase 3 and prevents their activity (Zhou et al., 2023).

BCL-2 family proteins are significant factors in the regulation of the apoptosis pathway. The evidence has revealed that the low expression of proapoptotic BCL-2 family, including BAX and BAK, is correlated with drug resistance in melanoma, colon and ovarian cancer cells as well as acute myeloid leukemia to docetaxel, cisplatin, and idarubicin (Guttà et al., 2020; Wang et al., 2022a). Additionally, high expression of antiapoptotic BCL-2 proteins (e.g., BCL-2, BCL-xl, MCL-1), decoy receptors, cellular FLICE-like inhibitory protein (c-FLIP), and inhibitor of apoptosis proteins (IAPs) can also promote resistance to chemotherapy due to apoptosis resistance (Dogan et al., 2022).

The intracellular cell death program is regulated by multiple signaling cascades and various transcription factors, cytokines, and growth factors including NF-κB, Apaf-1, STAT3, P53, c-Myc, and survivin. NF-κB is a key protein in the modulation of apoptosis and has a prosurvival function in apoptosis response. NF-κB signaling inhibits cancer cell apoptosis by upregulating antiapoptotic molecules, such as Bcl-2, Bcl-XL, and FLIP, and reducing the levels of reactive oxygen species (ROS) in tumor cells by increasing the transcription of some antioxidant enzymes, which leads to therapeutic resistance in various tumors (Wu et al., 2023a). Unlike NF-κB, the oncosuppressor p53 is one of the key transcription factors in the induction of apoptosis in response to DNA damage caused by chemotherapy. The role of p53 in promoting MDR may be also due to its inhibitory effect on the transcription and expression of some MDR genes. Therefore, mutation and inactivation of p53 attenuate the sensitivity of cancer cells to chemotherapeutic agents (Zhu et al., 2020).

Besides the loss of function mutation of p53, many cancers carry a specific type of p53 mutation, which acquired oncogenic properties known as "gain of function" (GOF). The mutant

p53 GOF facilitates tumor progression, metastasis, and therapy resistance (Alvarado-Ortiz et al., 2021). Recently Vogiatzi et al. (2016) have shown that a GOF activity of mutp53 mediates invasion and lung metastasis of pancreatic cancer via ectonucleoside triphosphate diphosphohydrolase 5 (ENTPD5) gene targeting and knockdown of p53 GOF reduced invasive potential of cancer cells. Similarly, Phatak et al. (2021) have found mutant p53 GOF could promote invasion, metastasis, and chemoresistance mediated by Rab-coupling protein (RCP). They noted that in cancer cells with p53 GOF mutation, there is increased interaction between RCP and P-gp contributing to an increase in P-gp localization in cell membrane in response to cisplatin and etoposide.

Survivin, an inhibitor of apoptosis (IAP) family member, has a central role in cell division and apoptosis suppression. The high expression of survivin promotes cell growth and confers chemoresistance by apoptosis evasion in cancer cells (Albadari and Li, 2023).

The two common therapeutic strategies to override apoptosis include stimulation of proapoptotic proteins or inhibition of antiapoptotic proteins, and activation of caspase enzymes. The drug development research has focused on a variety of targets that stimulate apoptosis such as cell death receptor ligands, alkyl phospholipid (ALP), and analog inhibitors for BCL-2 and XIAP (Dakir et al., 2023; Hashimoto et al., 2021). For example, ABT-737 and ABT-199 are small molecules that inhibit BCL-2 and BCL-xL with high specificity and selectivity, which can induce apoptosis. Furthermore, the overexpression of caspase inhibitors (XIAP and c-Flip) leads to the evasion of apoptosis in cancer cells. According to this, a group of drugs called "SMAC mimetic" have been developed that destroys the interaction between XIAP and active caspases (Bai et al., 2014). In addition, DEBIO-1143 is an orally available antagonist of inhibitor of apoptosis proteins (cIAP1/2 and XIAPs). This antagonist is an SMAC mimetic-based treatment in a phase II clinical trial with the potential to reverse carboplatin resistance in ovarian cancer, and cisplatin resistance in head and neck carcinoma by targeting XIAPs and apoptosis induction (Sun et al., 2020; Tao et al., 2023).

As a result, advancing our understanding of the cell death program process in cancer cells has led to the identification of key regulatory factors. Therefore, reactivation of apoptosis for overcoming chemoresistance has gained the attention of researchers as a promising new intervention in cancer therapy.

1.6 Cancer stem cells and epithelial-mesenchymal transition

Cancer stem cells (CSCs) or tumor initiating cells (TICs) are a group of multipotent cells that are responsible for tumor initiation, drug resistance, and cancer relapse due in part to their self-renewal capability and differentiate into the heterogenous cancer cell lineages. CSCs are also able to induce cell cycle arrest (quiescent state), which enables them to develop resistance against chemo- and radiotherapy (Lee et al., 2020). Moreover, it is known that some populations of CSCs share EMT-like cell features, which are often associated with resistance to apoptosis, acquisition of tissue invasiveness, and cancer drug resistance. For instance, it has been reported that the upregulation of AXL, the EMT-associated gene, can predict the acquired resistance to EGFR-TKI osimertinib. The proposed molecular mechanism for this drug resistance involves cell stem characteristics, the proapoptotic protein Bcl-2-like protein 11, or the chromatin remodeling induced by EMT-related transcription factors (EMT-TFs) (Chamorro et al., 2023; Ohara et al., 2021).

EMT is an extremely dynamic and reversible process that enables epithelial cells to acquire mesenchymal features such as increased cell motility and invasiveness, metastatic colonization, as well as resistance to apoptosis cell death. EMT occurs during some biological processes such as embryonic development and organogenesis, adult tissue regeneration, wound healing, and tissue repair. Recently, it has been shown that it also plays a crucial role in cancer progression and therapy resistance by inducing stem cell properties, inhibiting apoptosis, senescence, and contributing to immunosuppression (Ribatti et al., 2020). EMT is primarily regulated by signals from the microenvironment including inflammation, growth factors, cytokines, hypoxia, and pH. These upstream signals modulate important EMT-associated transcription factors that regulate the cellular phenotype and plasticity of tumors in response to changes in the microenvironment and the progressive nature of cancer (Wang et al., 2022b).

Several signaling pathways such as TGF-β, Wnt, Hedgehog, Notch, NF-κB, mitogen-activated protein kinase (MAPK or MAP kinase), and phosphatidyl inositol 3-kinase (PI3K) signaling are involved in EMT regulation in cancer cells, which are also common in CSCs (Deshmukh et al., 2021). Eventually, these pathways will lead to stimulation of EMT-TFs including Snail, zinc-finger E-box-binding (ZEB), and the basic helix−loop−helix (bHLH) family (Twist1 and Twist2). EMT-TFs repress the expression of E-cadherin and other epithelial markers (e.g., desmoplakin and claudins) and induce the expression of genes associated with the mesenchymal phenotype (e.g., N-cadherin, vimentin, fibronectin, and matrix metalloproteinases [MMPs]) (Buyuk et al., 2022).

EMT is a tightly regulated process due to the significant consequences that may arise from any abnormal transition, especially in the context of cancer. Noncoding miRNAs, posttranslational regulation, as well as epigenetic modifications are also involved in the regulatory network of EMT (Huang et al., 2022). Noncoding miRNAs can either downregulate EMT-TFs or act as their functional mediators in the regulation of the EMT process. For example, the discovery of miRNAs that target important signaling pathways (e.g., TGF-β, Notch, and Wnt signaling pathways) in EMT and cancer stem cells showed the existence of an EMT promoting crosstalk between these pathways. Researchers indicated that miR-3, miR-153, and miR-363 enhanced chemosensitivity by Snail targeting (Dong et al., 2021). EMT-TFs can be regulated through epigenetic modification. For instance, EMT-TFs recruit histone-modifying enzymes to repress CDH1 promoter. CDH1 gene encodes E-cadherin (Huang et al., 2022).

Furthermore, posttranscriptional and posttranslational modifications as well as epigenetic alterations can modulate the EMT process by regulating the expression of related EMT-TFs, which are correlated to drug resistance in cancer cells via different molecular mechanisms. In esophageal cancer, HOTAIR—a long noncoding RNA that sponged several miRNAs—sponges miR-148a that lead to positive regulation of EMT-TF Snail 2 and promoting of EMT, metastasis, and chemoresistance (Amicone et al., 2023). Therefore, targeting EMT-related TFs is emerging as a promising strategy to combat metastasis and chemotherapy resistance.

The aforementioned are some of the common examples of the numerous mechanisms involved in cancer chemoresistance. Many researches have also been carried out to elucidate the involvement of other mechanisms and molecules such as mitochondrial alteration, tumor microenvironment (TME) interactions, drug metabolisms in the tumor, microRNAs (miRNAs), small interfering RNAs (siRNAs), and their contribution to chemoresistance (Wang et al., 2020).

2. Matrix metalloproteinases

The extracellular matrix (ECM) is not only responsible for providing structural support in organs and tissues, but is also involved in various physiological processes, such as regulating the cell cycle, cell motility, survival, and apoptosis. It also helps to distribute growth factors and incorporate signals into the cells (Cabral-Pacheco et al., 2020). The ECM is a complex assembly of hundreds of different molecules, including glycosaminoglycans, proteoglycans, structural and adhesion proteins, as well as proteolytic enzymes known as MMPs. MMPs comprise a group of 23 endopeptidases in human. They are named for the presence of chelated zinc and calcium in their structures and can cleave and remodel the ECM proteins. MMPs can degrade all types of ECM components, except for polyglycan (Bandzerewicz and Gadomska-Gajadhur, 2022).

MMPs can be classified into secreted and membrane type-MMPs (MT-MMPs) according to their architectural features. MT-MMPs include type I transmembrane-type MMPs (MT1-MMP, MT2-MMP, MT3-MMP, and MT5-MMP), glycosylphosphatidylinositol-linked MMPs (MT4-MMP and MT6-MMP), and a type II transmembrane-type MMP (MMP-23). Similarly, based on their specificity for ECM components and the organization of their structural domains, secreted MMPs are further divided into collagenases (MMP-1, -8, -13, and -18), gelatinases (MMP-2 and MMP-9), stromelysins (MMP-3, -10, and -11), matrilysins (MMP-7), and other MMPs (Cabral-Pacheco et al., 2020).

MMPs are synthesized in an inactive form known as zymogens or pro MMPs, which are activated by various proteinases including other MMPs or free radicals through the cysteine switch mechanism. Generally, MMPs play a key role in various physiological and pathological processes, including tissue remodeling, organ growth and development, regulation of inflammatory processes, cell migration, and cancer progression that are regulated by different growth factors, cytokines, and hormones (Almutairi et al., 2023). MMPs expression in normal tissues is tightly controlled, and they present in low expression, unlike in cancer tissues. The local balance between MMPs and their physiological inhibitors is what determines their function in vivo. The most important endogenous inhibitors of MMPs' function are the tissue inhibitors of metalloproteinases (TIMPs), which are also frequently expressed in tumors. TIMP-1, -2, -3, and -4 form complexes with active MMPs, leading to inhibition of proteolytic activity of these enzymes (Tune et al., 2022). The implication of MMPs in tumor progression, migration, and metastasis through EMT induction has been reported in many cancers including lung, colorectal, gastric, pancreatic, ovarian, and prostate cancer (Dai et al., 2021; Loret et al., 2019; Odero-Marah et al., 2018). The ability of MMPs to activate other MMPs can create a positive feedback loop. The intricate interplay in MMPs leads to a cascade of activation and amplifies their impact on the EMT process (Almutairi et al., 2023).

There are three distinct mechanisms by which MMPs are linked to EMT in cancer progression: firstly, increased levels of MMPs in the tumor microenvironment can trigger the EMT process in epithelial cells. Secondly, cancer cells that undergo EMT can produce more MMPs, which in turn facilitates their invasion and metastasis. Thirdly, EMT can generate activated stromal-like cells that promote the progression of cancer by increasing MMP production (Radisky and Radisky, 2010). For example, the presence of MMP-9, which originates from the tumor microenvironment, tumor cells, and stromal cells adjacent to the tumor, facilitates the EMT process through the TGFβ1 signaling cascade, upregulation of Snail family,

cleavage of E-cadherin, and induction of mesenchymal markers expressions such as vimentin, fibronectin, and N-cadherin. As well, several MMPs (e.g., MMP-3, -7, -9, -10, -12, and -14) are involved in the regulation of the Wnt/β-catenin signal pathway that plays an important role in the induction of EMT. Additionally, membrane type-1 matrix metalloproteinase (MT1-MMP) was confirmed to play a significant role in inducing EMT by regulating the expression of EMT-associated genes including vimentin, N-cadherin, Snail, Slug, and ZEB1 in various cancers. In further investigation, it was also found that increased activity of MMP-2, -9, and -13 correlated with overexpression of MT-MMP1 in gastric cancer (Li et al., 2015). Noteworthy, dysregulation of MMPs also contributes to the induction of chemoresistance as a consequence of their involvement in the ECM degradation, EMT promotion, apoptosis resistance, and interference with Akt, EGFR, and MAPK signaling pathways that are implicated in EMT process. Other possible mechanisms such as inhibition of cell cycle arrest and Fas cleavage are also involved in inducing chemoresistance by MMPs (Tune et al., 2022). Furthermore, MMPs are involved in the upregulation of EMT-TFs via the Wnt/β-catenin pathway and ROS production that suppresses E-cadherin expression (Huang et al., 2022). Previous report demonstrates that MMP-3 stimulates Rac1b expression, a variant of Rac1, which localizes in the plasma membrane and is related to ROS production by NADPH oxidase, ultimately leading to the upregulation of Snail (Melzer et al., 2019). The network of signaling pathways dependent on EMT-TFs is essential for EMT-induced chemoresistance. Previous reports revealed that increased expression of Snail resulted in upregulation of P-gp in breast cancer and NSCLC, which can promote resistance to cisplatin, doxorubicin, and 5-FU (Seo et al., 2021). Likewise, temozolomide resistance in glioblastoma was mediated by NFE2L2/MMP-2 axis and overexpression of vimentin, Snail, and Slug (Rajesh et al., 2019). Moreover, the enhanced expression of mesenchymal markers, N-cadherin and β-catenin, as well as MMP-2, and MMP-9 and reduced expression of E-cadherin has been documented in doxorubicin-resistant breast cancer cells (Paramanantham et al., 2021). However, clinical studies that define the impact of MMPs in sensitivity to chemotherapy are conflicting, possibly depending on the type of MMP or cancer. For instance, higher MMP-2 expression was demonstrated in platinum-sensitive ovarian cancer patients compared to resistant and refractory tumors (Jeleniewicz et al., 2019). Similarly, in breast cancer patients treated with 5-FU, doxorubicin, and cyclophosphamide, tumor response was not influenced by changes in serum levels of MMP-2 and MMP-9 (Gonzalez-Avila et al., 2019).

All of these data support the role of MMPs in promoting EMT-dependent chemoresistance; therefore, the development of new therapeutic strategies to inhibit MMPs functions to overcome therapy resistance is a particularly attractive proposal. In the following, we discuss some of the accumulated evidence demonstrating the role of some types of MMPs in cancer therapy resistance.

2.1 Matrix metalloproteinase-1

Matrix metalloproteinase-1 (MMP-1) is identified as the most predominant form of collagenase enzyme that cleaves collagen types I, II, III, VI, VII, VIII, and X. In addition, MMP-1 breaks down other substrates such as gelatin, laminin, TNF, IL-1, and insulin growth factor–binding proteins. MMP-1 activates other MMP proenzymes, including MMP-1, -2, and -9, and normally are inhibited by TIMP-1 (Mondal et al., 2020).

Increased expression of MMP-1 has been implicated in various tumors and found to be closely correlated with early metastasis and poor prognosis. MMP-1 was also associated with the migration and invasion of tumor cells, most likely because of ECM degradation in the process of EMT. For example Wang et al. (2022a) have indicated that MMP-1 promoted the malignant progression of colorectal cancer through EMT and the PI3K/Akt signaling pathway. Moreover, this enzyme participates in the EMT process by regulating of NT-3/TrkC and Wnt signaling pathway.

Several studies have also indicated that high MMP-1 expression contributes to multidrug resistance in cancer. In a recent study, Kim et al. (2022) demonstrated that increased expression of MMP-1 by hypomethylation of MMP-1 promoter leads to induce tamoxifen resistance in breast cancer cells. Likewise, nasopharyngeal carcinoma' chemoresistance to 5-FU was mediated by MMP-1 upregulation, and silencing of MMP-1 restored the chemosensitivity of cancer cells, inhibited the growth of cancer cells, and increased apoptosis (Song et al., 2019). Furthermore, MMP-1 expression was reported to be upregulated in EGFR-TKI−resistant lung adenocarcinoma cells, more significantly than in EGFR-TKI−sensitive cells. It was also speculated that MMP-1 expression induced by the mTOR pathway plays an important role in regulating of migratory and invasive behavior of these cells, leading to poor prognosis (Xiao et al., 2016). Recently, it was shown that a miR-145-mediated downregulation of MMP1 in imiquimod-treated cells reversed the acquisition of stem cell−like properties and chemoresistance. This study suggested that MMP-1 can also increase the stemness of glioblastoma cancer cells, which may be another possible mechanism for MMP-1-induced chemoresistance (Zhu et al., 2022).

2.2 Matrix metalloproteinase-2

Matrix metalloproteinase-2 (MMP-2) is a kind of gelatinase that degrades various ECM proteins such as gelatin, collagen types IV, V, and XI, as well as many other substrates involved in ECM degradation. MMP-2 is another such key proteolytic enzyme among MMPs that its overexpression in most tumors is linked to chemotherapy resistance in addition to promoting tumor aggressiveness, angiogenesis, and metastatic potential (Bibak et al., 2019). High MMP-2 levels have been reported to correlate with tumor metastasis and reduced survival in cervical cancer, renal cell carcinoma, breast cancer, and glioma (Ramachandran et al., 2017). Moreover, it has been found that there is a positive correlation between the expression of MMP-2 and MMP-9 and tumor stage, as well as lymph node metastasis in breast cancer. Furthermore, it was suggested that these two enzymes can serve as reliable prognostic factors for breast cancer (Li et al., 2017). Similar results were reported by Song et al. (2021), who indicated that MMP-2 and MMP-9 expression was increased in hypopharyngeal carcinoma, and therefore, they may be regarded as key positive factors for tumor development, invasion, and metastasis.

The possible proposed mechanisms of chemotherapy resistance mediated by MMP-2 include the promotion of EMT, apoptosis inhibition, and cell proliferation activation, which may have the ability to counteract the effects of chemotherapeutic drugs. For example, overexpression of MMP-2, vimentin, and Snail (EMT-TF) contributes to the triggering of notch signaling pathway that promotes EMT-induced chemoresistance (Tune et al., 2022). As well, there is an interaction between Snail and several MMPs such as MMP-2, -3, -7, -9, and -14

that contribute to the EMT process (Jin et al., 2010; Lin et al., 2011). In breast cancer cells, the activation of Akt/ERK signaling pathway by MMP-2 and MMP-9 leads to epirubicin resistance. Interestingly, these two enzymes are also engaged in STAT3 signaling activation, which leads to the invasiveness and multidrug resistance of breast cancer cells (Zhang et al., 2015). Similarly, overexpression of MMP-2 has been reported in breast cancer cells resistant to doxorubicin, adriamycin, and tamoxifen (Paramanantham et al., 2021; Park et al., 2019). Likewise, resistance to vemurafenib increases the invasiveness of melanoma cancer cells and modulates the TME by upregulating MMP-2. It was found that vemurafenib resistance mediated by MMP-2 involved the reactivation of MAPK pathway (Dratkiewicz et al., 2020). Moreover, the upregulation of MMP-2, MMP-9, and ERK/MMP signaling pathways has been reported in cisplatin-resistant muscle invasive bladder cancer that is mediated by overexpression of Derlin1 (Dong et al., 2017). MMP-2 expression was also reported to be upregulated in EGFR-TKI-resistant lung adenocarcinoma cells, more significantly than in EGFR-TKI-sensitive cells (Deng et al., 2020). Furthermore, there is evidence that MMP-2 is the most unregulated MMPs in glioblastoma (GBM) and in temozolomide (TMZ)-resistant GBM. NFE2L2, the master antioxidant transcription factor, has been found to regulate MMP-2 expression; therefore, the blockade of the NFEL2/MMP-2 axis prevents the acquisition of TMZ resistance in GBM cells by stimulating apoptosis and suppressing EMT (Rajesh et al., 2019).

2.3 Matrix metalloproteinase-3

Matrix metalloproteinase-3 (MMP-3) is a member of the stromelysin family, which can degrade noncollagen components of ECM including laminin, fibronectin, and proteoglycan. Furthermore, it digests growth factors, cell membrane receptors, and adhesion molecules. This enzyme can activate a variety of pro-MMPs, such as pro-MMP-1 and pro-MMP-9 (Wan et al., 2021). Therefore, the expression and activation of MMP-3 is the first step in the destruction of ECM by MMPs. MMP-3 participates in multiple cell biological processes such as inflammation, cell differentiation, and disease progression. Moreover, MMP-3 is significantly upregulated in multiple cancers and regulates important signaling pathways including apoptosis, cell growth, invasion, metastasis, and chemoresistance. Given the crucial role of MMP-3 in the initiation of EMT in tumor cells, this enzyme can promote migration and invasiveness and enhance resistance to chemotherapy. MMP-3 and MMP-7 directly cleave E-cadherin at the cell surface and reduce cell—cell interaction, which causes cell invasion in a paracrine manner (Zhao et al., 2021). MMP-3 also plays a role in regulating the Wnt pathway by cooperating with Wnt3a to promote Wnt-induced EMT, enhance Wnt-mediated signaling, and increase the transcriptional activity of β-catenin (Gonzalez-Avila et al., 2019). Moreover, overexpression of MMP-3 induces EMT by upregulating Rac1b and Snail expression via ROS (Melzer et al., 2019).

In the context of chemoresistance, MMP-3 expression was reported to contribute to cancer invasion and poor response to gemcitabine in pancreatic cancer. Recently, Deng et al. (2022) have reported that a high level of MMP-3 expression was associated with gemcitabine resistance and cancer invasion in tissue samples of diabetic pancreatic ductal adenocarcinoma (PDAC) patients and reported that MMP-3/ROS signaling pathway involved in cancer invasion and gemcitabine resistance under high glucose condition. Further, MMP-3 expression was found to be closely associated with RRM1, a gene involved in gemcitabine metabolism. The previous investigation reported that miR-21 was involved in the activation of cancer-associated fibroblasts (CAFs) and

contributed to gemcitabine resistance in PDAC by overexpression of MMP-3 and MMP-9 (Zhang et al., 2018). As well, in cisplatin-resistant ovarian cancer, miR-18a-5p inhibits tumor growth by targeting MMP-3. Silencing of MMP-3 reduced cell growth, viability, and invasion of cisplatin resistance ovarian cancer cells (Diaz et al., 2020). Moreover, it was found that the MMP-3 polymorphism had a significant impact on the response to 5-FU-cisplatin neoadjuvant chemotherapy in head and neck carcinoma (Blons et al., 2004).

2.4 Matrix metalloproteinase-7

Matrix metalloproteinase-7 (MMP-7) (matrilysin) is the smallest MMPs that digests various ECM proteins such as collagen, proteoglycans, fibronectin, and laminin and also cleaves non-ECM substrates, such as E-cadherin and Fas ligand (FasL). The cleavage of E-cadherin is a crucial proteolysis activity of MMP-7, that leads to the production of soluble E-cadherin (sE-cad) (Jang et al., 2016). SE-cad mediates invasive phenotype in tumor cells through the induction of MMP-2, -9, and -14 expressions (Nawrocki-Raby et al., 2003).

As with other MMPs, MMP-7 plays a vital role in regulating multiple signaling pathways and processes such as, cell growth, inflammation, angiogenesis, wound healing, and aging. MMP-7 also regulates cancer development, invasion, and metastasis in several types of cancer cells through various mechanisms, including inhibition of apoptosis, progression of EMT, reduction of cell adhesion, and angiogenesis induction (Liao et al., 2021). Besides, MMP-7 activates MMP-1, MMP-2, MMP-8, MMP-9, and MMP-13 zymogens that contribute to tumor progression indirectly (da Silva-Neto et al., 2022; DeLeon-Pennell et al., 2017).

MMP-7 plays a crucial role in the regulation of the EMT process by cleavage of E-cadherin. For instance, MMP-7 induces syndecan-2 proteolytic degradation and shedding of E-cadherin to promote EMT in colon cancer cells (Jang et al., 2016). There is also some evidence regarding the regulation of MMP-7 transcription by β-catenin, the main downstream mediator of Wnt signaling, in prostate cancer, gastric cancer, and oral squamous cell carcinoma (Wang et al., 2021; Guo et al., 2022; Zhou et al., 2021b). In contrast, MMP-7 is capable of regulating β-catenin function and releasing β-catenin from the E-cadherin/β-catenin complex in prostate cancer cells (Wang et al., 2021). Additionally, in hepatocellular carcinoma, MMP-7 is involved in EMT and metastasis by regulating the MMP-7/syndecan-1/TGFβ1 autocrine loop (Zeng et al., 2016).

Other than that, several studies have found that overexpression of MMP-7 is a contributing factor to multidrug resistance in many cancers. The MMP-7 enzyme is known to have an interaction with the Fas/FasL system. MMP-7 regulates Fas expression and activation and generates soluble Fas by cleaving its membrane form, the Fas receptor, as well as its ligands. Therefore, MMP-7 can confer protection to tumor cells against the cytotoxic effects of chemotherapeutic drugs by blocking the induction of apoptosis by Fas activation (Mitsiades et al., 2001). Consistent with this, MMP-7 could increase oxaliplatin resistance by decreasing the Fas receptor in colon cancer cells (Almendro et al., 2009). MMP-7 has also been reported to promote tumor survival and resistance to doxorubicin by cleaving FasL and decreasing its effectiveness in triggering Fas-mediated apoptosis (Mitsiades et al., 2001). Mohan and coworkers (Mohan et al., 2021) have reported that the use of antiMMP-7 monoclonal antibodies can stabilize Fas ligand, arrest migration, and sensitize ductal pancreatic adenocarcinoma cells to gemcitabine and oxaliplatin. In addition, studies have reported the role of MMP-7 in acquired chemoresistance against anthracycline and taxane by degrading IGF-binding protein-3

(IGFBP-3) in advanced colorectal cancer (Tune et al., 2022). Moreover, the nucleolar assembly and intranucleolar cleavage of p53 in gefitinib-resistant cancer stem cells was mediated by MMP-7. This study suggested that the MMP-7-MUC-1-p53 axis in nucleolus could be a promising therapeutic target for anti-CSCs to address the chemotherapy-resistance dilemma (Yu et al., 2020). The role of MMP-7 in platinum resistance is also being supported by a growing body of evidence, which facilitates the escape of tumor cells from chemotherapy-induced apoptosis. For instance, the elevated level of MMP-7 in serum and tissue samples of patients was correlated to poor survival outcomes and resistance to platinum-based cancer therapy in bladder cancer, non−small cell lung cancer, and castration-resistant prostate cancer (Liu et al., 2008; Szarvas et al., 2021). Furthermore, the prognostic value of MMP-7 in response to various anticancer drugs has been validated in several studies. In a recent study, MMP-7 was identified as a potential serum marker in predicting survival and response to docetaxel, abiraterone, and enzalutamide therapy in patients with prostate cancer (Szarvas et al., 2021).

2.5 Matrix metalloproteinase-9

Matrix metalloproteinase-9 (MMP-9), also known as gelatinase B and type IV collagenase, is one of the most important and investigated MMPs that play vital roles in various biological processes. In addition to the degradation of many ECM proteins, it can also degrade some cell surface and intracellular proteins (Luchian et al., 2022). Due to the significant role of MMP-9 in ECM remodeling, cleavage of the basement membrane, and EMT induction, its association with cancer-related processes, such as angiogenesis, migration, invasion, metastasis, and chemoresistance, has been proposed by many studies (Barillari, 2020). Therefore, some recent advances point to this enzyme as a potential biomarker for different cancers. Tumoral MMP-9 overexpression is positively linked to a poor prognosis in colorectal, breast, ovarian, and non−small cell lung cancer (Jafarian et al., 2020; Kwon, 2023; Peltonen et al., 2021; Wu et al., 2023a,b).

A study investigates the significance of MMP-9 as a key driver of EMT in thyroid cancer cells and found that EMT is unable to proceed without MMP-9 activity. The study further revealed that knocking down MMP-9 inhibited TGF-β1-mediated EMT, as evidenced by the increase in E-cadherin expression and decrease in vimentin expression. Therefore, EMT-induced chemoresistance is directly linked with the overexpression of MMP-9 (Li et al., 2020).

Recent research demonstrated that upregulation of MMP-9 results in the incidence of more aggressive phenotype of cancer cells, metastasis, and relapse in breast cancer, colorectal cancer, gastric cancer, and ovarian tumors (Prathipaa et al., 2021; Kwon, 2023; Peltonen et al., 2021; Wu et al., 2023b). MMP-9 is also crucial in conferring resistance to various cancer therapies by, but is not limited to, modifying tumor vasculature to impact drug delivery, promoting cancer cell survival, or inducing EMT-related processes (Wang et al., 2023).

Golus et al. (2022), have shown that STAT3 signaling pathway contributes to the EMT induction and chemoresistance in bladder cancer via the upregulating of MMP-9. Further, in cisplatin-resistant ovarian cancer cells with elevated expression of MMP-9, administration of MMP-9/MMP-2 inhibitors before cisplatin indicates a chemosensitizing effect as evident by enhancing cisplatin-induced cell death (Laios et al., 2013).

Recently, the special AT-rich sequence binding protein 1 (SATB1) was implied to contribute to radiation and cisplatin resistance in nasopharyngeal carcinoma cells by MMP-9 upregulation and EMT promotion (Zhou et al., 2021a). MMP-9 overexpression

was also implied to contribute to poor response to oxaliplatin in gastric cancer by upregulating VEGF. The expression of VEGF may be affected indirectly by MMP-9 expression through the inhibition of E-cadherin. Decreased expression of E-cadherin leads to activation of ERK/Akt pathway, which results in VEGF upregulation (Gao et al., 2017). Another study showed that suppressing miR-211 and/or MMP-9 expression could lead to a decrease in P-gp efflux in glioma cancer cells (Asuthkar et al., 2012). As well, in osteosarcoma cancer cells, microRNA-29b enhances the sensitivity of cancer cells to doxorubicin by specifically targeting MMP-9 (Luo et al., 2019).

2.6 Matrix metalloproteinase-14

Matrix metalloproteinase-14 (MMP-14) is a membrane-anchored MMP (MT1-MMP), which has both proteolytic and nonproteolytic functions. In addition to cleavage of collagen, gelatin, vitronectin, and laminin 1, it can activate proMMP-2, -9, and -13. MMP-14 is known to play a critical role in cancer cell migration, invasion, metastasis, and resistance to chemotherapeutic agents through ECM degradation, pro-MMP-2 activation, and EMT promotion (Knapinska and Fields, 2019).

Moreover, it can regulate angiogenesis, which is another hallmark of cancer. However, it was later reported that MMP-14 activity has a negative impact on immune responses to cancer (Tanaka and Sakamoto, 2023). Data supporting the involvement of MMP-14 in different types of cancer through regulating EMT has been provided by several investigators. It was demonstrated that MMP-14 is overexpressed in various cancers, including synovial sarcoma, prostate cancer, ovarian cancer, gastric cancer, and breast cancer, and confers EMT (Carey et al., 2021; Gonzalez-Molina et al., 2019; Robert and Paul, 2017; Wang et al., 2021). MMP-14 can facilitate EMT through regulation of EMT markers expression, for instance, N-cadherin, vimentin, twist, ZEB1, and E-cadherin, activation of TGF-β signaling, and upregulation of Wnt5a (Djediai et al., 2021; Knapinska and Fields, 2019).

In the context of chemoresistance, a strong correlation between the high level of MMP-14 expression and the emergence of chemoresistance has been revealed in cancers. For example, MMP-14 upregulation contributes to poor response to chemotherapy in triple-negative breast cancer, and its inhibition improves sensitivity to radiation and doxorubicin in this cancer (Thakur et al., 2019). MMP-14 has also been reported to play a crucial role in the mechanism of chemoresistance in GBM. The suggested explanation for how MMP-14 contributes to chemoresistance involves its impact on DNA double-strand breaks. When MMP-14 expression is suppressed, the DNA damage marker γH2AX becomes more phosphorylated. This suggests that overexpression of MMP-14 may protect cells from apoptosis by decreasing the amount of DNA damage caused by chemotherapy and radiation therapy (Rajesh et al., 2019). Likewise, MMP-14 can protect pancreatic cancer cells from DNA damage and cell cycle arrest-induced gemcitabine by phosphorylation of ERK1/2 and increased expression of high mobility group A2 (HMGA2) (Gu et al., 2021). Moreover, MMP-14 confers radio/chemoresistance by regulating the stability of laminB1, a large family of intermediate filaments, in replication fork and reducing DNA damage in breast cancer cells. On the other hand, depletion of MMP-14 induces DNA replication stress and double-strand breaks by contracting integrin b1 signaling (Thakur et al., 2021). Aside from its role in DNA damage response, a comparative secretum analysis in pancreatic ductal adenocarcinoma (PDAC) demonstrated that MMP-14 is a secreted

major factor in gemcitabine-resistant PDAC cells. Furthermore, the MMP-14-carrying exosome could mediate drug resistance and migratory phenotype in sensitive PDAC cells (Li et al., 2022).

2.7 Other MMPs with possible involvement in cancer chemotherapy resistance

The importance of MMPs in ECM degradation makes them a crucial player in EMT, which contributes to chemoresistance mechanisms. In addition to this, the involvement of other mechanisms such as inhibition of apoptosis, interference with different cell signal pathways, and induction of cell cycle arrest in inducing and regulating cancer chemoresistance have also been clarified. As discussed earlier, some MMPs are more prominently involved in chemotherapy resistance. At the same time, there are many additional MMPs, we have not highlighted, that have gained substantial attention during recent years due to their potentially important roles in cancer chemoresistance (Table 23.1). There are also other MMPs that play a key role in various physiological and pathological processes, but their involvement in chemoresistance is not reviewed yet. Further research is needed to fully understand the specific mechanisms by which these MMPs influence chemoresistance and to determine their potential as therapeutic targets for improving cancer treatment outcomes.

TABLE 23.1 Specific MMPs and their proposed mechanisms in chemoresistance.

MMP	Cancer type	Biological effect	Mechanisms	References
MMP-8	Lung adenocarcinoma Kidney cancer	Resistance to EGFR-TKI Sunitinib resistance	Not mentioned	Finke et al. (2011), Saito et al. (2018)
MMP-10	Ovarian cancer	Resistance to platinum-based drugs Cisplatin resistance	Wnt signaling	Mariya et al. (2016), Solár and Sytkowski (2011)
MMP-11	Prostate cancer Breast cancer	Resistance to androgen deprivation therapy Lapatinib resistance	miR-135a miR-153-p/ANLN axis	Wan et al. (2016); Wu et al. (2021)
MMP-12	Lung cancer	Gefitinib resistance	EGFR/ERK/MMP-12 pathway	Xiao et al. (2016)
MMP-13	Gastric cancer	Cisplatin resistance	LINC00332[a]	Taghehchian et al. (2022)
MMP-17	Triple-negative breast cancer	Erlotinib and palbociclib[b] resistance	Not mentioned	Foidart et al. (2019)
MMP-19	Ovarian cancer	Resistance to A-1210477[c], vincristine, and carboplatin	Not mentioned	Wang et al. (2019)
MMP-20	Ovarian cancer	Resistance to A-1210477[c], vincristine, and carboplatin	Not mentioned	Wang et al. (2019)

[a]*Long intergenic nonprotein coding RNA 332.*
[b]*Anti-CDK4/6 inhibitor.*
[c]*Selective MCL-1 inhibitor.*

3. Conclusion and future perspectives

Cancer is one of the leading causes of human death worldwide. Although recent advances in cancer treatment resulted in better overall survival rates, drug resistance, tumor recurrence, and metastasis are still significant challenges to successfully treating cancer patients. In light of the rapid increase in cancer patient populations that may develop tumor resistance, there is a need to further study and understand the potential drug resistance mechanisms to develop new drugs to combat chemotherapy resistance. In addition to drug efflux mechanisms, the presence of CSCs, genetic mutations and epigenetic alterations, increased DNA repair capacity, apoptosis inhibition, cancer stemness, and EMT have been identified as potential factors that contribute to the occurrence of chemoresistance.

MMPs have an important role in promoting cancer progression, metastasis, and chemotherapy resistance. Their role in chemoresistance is mainly related to their involvement in ECM degradation and EMT induction, apoptosis inhibition, cell cycle arrest inhibition, and interference with different signaling pathways. Therefore, they are an attractive target for the development of novel cancer therapies and the stimulation of chemosensitivity to enhance cancer treatment. Nonetheless, there is still a need for further investigations to utilize this knowledge effectively. Additionally, by increasing our knowledge in this field, we can develop and work on novel and alternative strategies for reducing drug resistance and enhancing the chemotherapeutic response.

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CHAPTER

24

MMPs (-2 and -9)-mediated drug resistance and their reversal in gynecological cancer

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Abbreviations

AATF Apoptosis antagonizing transcription factor
CSCs Nature of cancer stem cells
ECM Extracellular matrix
PMA Phorbol 12-myristate 13-acetate
TIMPs Tissue inhibitors of metalloprotease
TME Tumor microenvironment
VEGF Vascular endothelial growth factor

1. Introduction

Obesity currently represents a global pandemic, with escalating trends observed worldwide (Opoku et al., 2023). Obesity emerges as a pivotal risk factor in the onset of gynecological cancers (GyCs), encompassing endometrial (EC), cervical (CC), as well as ovarian (OC) cancers (Wichmann and Cuello, 2021). Consequently, lifestyle interventions are crucial in enhancing patient outcomes for obesity-related GyCs. They are most prevalent among female cancers and pose a substantial public health challenge. They persist as a significant contributor to cancer mortality globally (Liontos et al., 2022). Owing to lack of efficacious treatment strategies, many patients with advanced GyCs resist conventional therapies, resulting in a poor prognosis (Song and Zhang, 2022). Further, GyCs are intricate and heterogenous diseases (Dwivedi et al., 2021). Regardless of extensive research, the molecular mechanisms governing their pathology and development remain elusive.

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Globally, EC ranks as the most prevalent GyC in high-income countries (Crosbie et al., 2022). EC exhibits heterogeneity with varied molecular subtypes and prognoses. Traditionally, EC has been grouped into type I mainly consisting of low-grade tumor cells with favorable prognosis, driven by estrogen, primarily endometrioid adenocarcinomas; and type II, consisting of high-grade cells with an unfavorable prognosis, including poorly differentiated carcinosarcomas (Makker et al., 2021). Generally, advanced age, overweight, no marriage, and the use of hormone replacement therapy are recognized as risk factors for EC. Currently, there is no established screening protocol for early EC detection. Hysteroscopic endometrial biopsy is the sole technique enabling selective biopsy of specific areas within the endometrium (Carugno et al., 2021). MRI with contrast-enhancing agents is the mainstay for imaging assessment of EC (Maheshwari et al., 2022). The treatment of EC primarily involves surgery, with adjuvant radiation and chemotherapy tailored to the grade and stage of the disease (Passarello et al., 2019). Recently, the treatment landscape has been transformed by immunotherapy with checkpoint inhibitors. Notably, pembrolizumab/lenvatinib has gained approval for all EC patients (Marín-Jiménez et al., 2022).

OC ranks as the seventh most prevalent cancer in women and the eighth leading cause of cancer-related deaths, exhibiting 5-year survival rates below 45% on the global scale. Nevertheless, the incidence has remained stable over the past decade, displaying variations among countries and races. OC originates from epithelial, stromal, and germline cells (Gaona-Luviano et al., 2020). The high-risk factors for OC include tobacco use, hormone replacement therapy, and a strong familial history of the disease (La Vecchia, 2017). Historically, blood-based biomarkers such as carbohydrate antigen 125 (CA125) have been extensively utilized for OC screening (Zhang et al., 2021). More recently, recommended methods for early OC diagnosis include gene chips, proteomics, methylation profile-based approaches, and immunohistochemistry (Terp et al., 2023). The management approach for OC comprises surgery combined with platinum and taxane chemotherapy (Orr and Edwards, 2018).

Globally, CC ranks as the fourth most prevalent cancer among women. Nevertheless, there are geographic variations in the incidence of CC (Buskwofie et al., 2020). The variants of human papillomavirus (HPV), such as type 16 and 18, are recognized as primary risk factors for CC. Additionally, cofactors also impact the risk of HPV transmission as well as its progression to CC (Pimple and Mishra, 2022). These cofactors encompass long-term use of oral contraceptives, high parity, consumption of tobacco, lifestyle factors, and dietary factors. CC screening methods have progressed from morphological examination to molecular testing. Additionally, HPV genotyping is prevalent technique that has gained widespread recommendations and global utilization (Zhang et al., 2020).

2. MMPs in gynecological cancers

The molecular landscape of GyCs exhibits extensive variations, contributing to both metastasis and drug resistance. The insidious spread involves a critical partner: a family of enzymes called matrix metalloproteinases (MMPs). MMPs are grouped as 25 enzymes, which tightly regulate biological function associated with the growth factors and signalings, which can remodel during disease states (Cabral-Pacheco et al., 2020). This remodeling is crucial for healing wounds, but when dysregulated, it can contribute to diseases such as cancer metastasis (Yadav et al., 2014).

MMPs degrade innumerable proteins, including those in the ECM, and influence key cancer hallmarks such as cell proliferation, migration, and angiogenesis (Padežnik et al., 2023). While their collagen-degrading abilities are well known, research has revealed broader functions in cancer as MMPs shape the tumor microenvironment, orchestrate angiogenesis, and even influence immune surveillance—ultimately dictating tumor growth and spread (Duan et al., 2020). MMPs 2, 9, and 14, alongside their inhibitors (TIMP-1 and -2) and a key angiogenesis promoter (VEGF-A), play a main role in metastasis of GyCs. Their interaction with the ECM paves the way for cancer cells to infiltrate nearby tissues, vessels, and lymphatics (Suarez et al., 2017). In EC, chronic estrogen levels trigger the production of IL-8, COX, and induction of VEGF, which mediates the activation of the ERK1/2 pathway to trigger the expression of MMP-2/9. MMPs also influence fat cell formation and blood gelatinase levels, adding further complexity (Michalczyk and Cymbaluk-Płoska, 2021). CC and OC, too, rely on MMPs for their aggressive spread. These enzymes dissolve the ECM scaffold, allowing cancer cells to invade freely, unhindered by tissue barriers. Understanding this intricate dance between MMPs and GyCs is key to developing effective treatments (Escalona et al., 2021).

2.1 MMP-2 and MMP-9 as targets of GyCs

Hypoxia often triggers metastasis in GyCs by transcriptionally inducing the expression of MMP-2 and MMP-9 (Fig. 24.1). Hypoxia enhances the synthesis of transcription factors, growth factors, and MMP-2 and MMP-9, which triggers the EMT along with the migration of endothelial cells through the ECM, creating the way for the formation of new blood vessels—a critical supply for tumor growth (angiogenesis) (Fares et al., 2020). However, MMPs have internal control as TIMPs to keep their activity in check and regulate sustained tumor growth. Increased MMP levels often correlate with the tumor grade, invasion, and even lymph node involvement. Evidence suggests that manipulating TIMP levels and using synthetic MMPs inhibitors have successfully reduced metastasis in various cancer types (Quintero-Fabián et al., 2019). The crucial role of MMPs in tumorigenesis is their involvement in modulating the TME and metastasis in various cancers. The expression and activities of MMPs are intricately regulated by TIMPs, noncoding RNAs as well as E3 ubiquitin ligases. Recognizing the pivotal role of MMPs in oncogenesis has led to their consideration as attractive targets for antitumor treatments. Various strategies, such as peptides, small-molecule inhibitors, monoclonal antibodies and natural compounds that targeting the MMP activity, and RNAi therapeutics, have been explored. Nevertheless, these approaches present challenges such as severe side effects, poor solubility, and minimal oral bioavailability (Liu et al., 2022).

Hypoxia induces metastasis in GyCs by upregulating the expression of MMP-2 and MMP-9. Hypoxia activates the transcription factor HIF-1α, which then joins with HIF-1β. They go into the nucleus and bind to the ARE region of the MMP-2 and MMP-9 genes, triggering their expression. MMP-2 stimulates endothelial cell migration and new blood vessel creation in the tumor region. Both MMP-2 and MMP-9 enhance epithelial-to-mesenchymal transition (EMT), which aids GyCs metastasis. Under normal conditions, HIF-1α is degraded by the proteasome, which inhibits the production of MMPs 2 and 9.

A study reported that eight out of ten OC cases are often detected at advanced stages. Surgery and platinum-based chemotherapy form the first line of treatment, but relapses are common. Understanding the factors within the TME, especially those promoting resistance, is key.

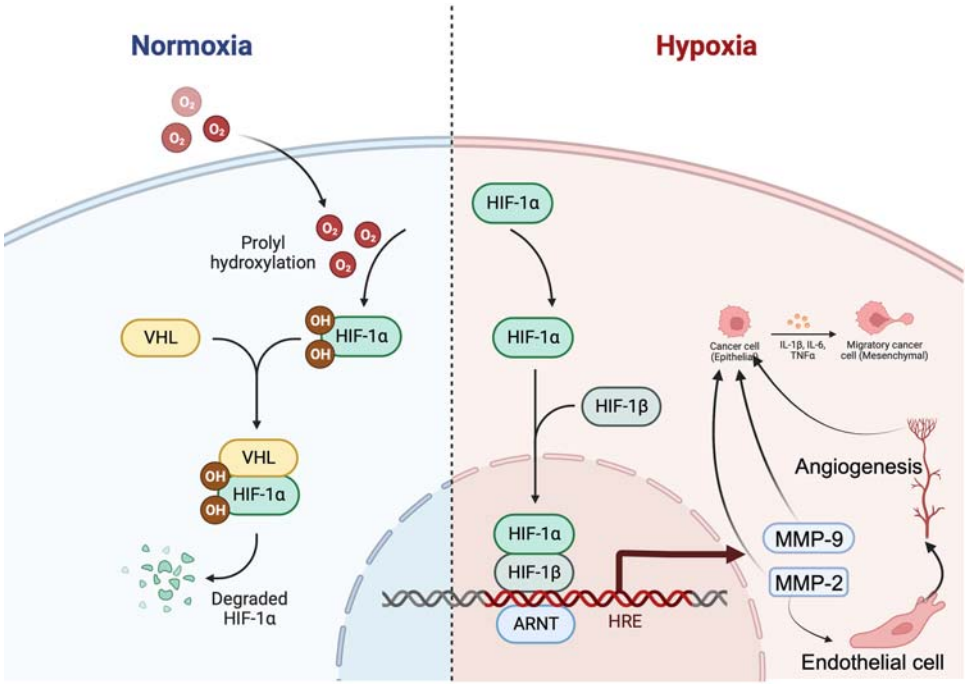

FIGURE 24.1 Hypoxia-induced metastasis mechanism in GyCs. Hypoxia promotes metastasis in GyCs by transcriptionally upregulating the expression of MMP-2 and MMP-9. Under hypoxic conditions, the transcription factor HIF-1α is activated and combines with HIF-1β. They translocate to the nucleus and bind to the ARE region of MMP-2 and MMP-9 genes, activating their expression. MMP-2 induces endothelial cell migration and new blood vessel formation in the tumor area. Both MMP-2 and MMP-9 promote epithelial-to-mesenchymal transition (EMT), facilitating GyCs metastasis. However, under normoxia condition, HIF-1α is under goes proteasome- mediated degradation and inhibits the MMP-2 and MMP-9 expression.

Imbalances in the MMP: TIMP ratio, particularly involving MMP-2, -9, -14, and TIMP-2, are linked to poorer survival. Ascites, the fluid collected in OC patients, become enriched with these MMPs, fueling the cancer's peritoneal spread. Intriguingly, chemotherapy treatments, mainly with cisplatin and paclitaxel (PTX), lead to increased TIMP-1 and -2 protein levels in OC cell lines, but TIMP-3 levels remain unchanged with increased MMP-2, -9, and -14 mRNA expression, along with the expression of cancer stem cell (CSC) markers associated with chemoresistance. Moreover, mutations in TIMP-2 or -3 favor chemotherapy response, while those in MMP-2 and -11 tip the scales toward resistance (Ozdemir et al., 2022).

MMP-2 and MMP-9 essentially involve in the metastasis of EC (Aglund et al., 2004), CC (Zhang et al., 2017), and OC (Che et al., 2015). The impact of various factors, such as cytokines, mitogens, inducers, and inhibitors, on the expression of MMP-2 and MMP-9 in CC (HeLa and DoTc2-4510) and OC (SK-OV-3) cancer cell lines was investigated in the past decade (Roomi et al., 2017). MMP-2 was expressed in HeLa and SK-OV-3 cell lines, while DoTc2-4510 cells expressed MMP-9. MMP-2 and MMP-9 are reported to express in RL95-2 and KLE cell lines (Liu et al., 2019). Treatment with phorbol myristate acetate (PMA) did

not affect MMP-2 expression in CC cell lines but moderately prompted it in the OC cell line SK-OV-3. MMP-9 was triggered by PMA in HeLa cells and augmented in DoTc2-4510. TNF-α and IL-1β slightly inhibited MMP-2 expression in HeLa cells, whereas lipopolysaccharide (LPS)-induced MMP-2 in HeLa cells. Conversely, inhibitors such as doxycycline, epigallocatechin gallate(EGCG), actinomycin-D (ActD), retinoic acid (RA), and dexamethasone (DEX) reduced the expression of MMP-2 in HeLa and SK-OV-3 cell lines and MMP-9 in DoTc2-4510. These findings highlight the modulatory effect of cytokines, mitogens, inducers, and inhibitors on the expression of MMP-2 and MMP-9 in OC and CC cells, suggesting their potential as effective strategies for treating these cancers (Roomi et al., 2010).

2.2 MMP-2 and MMP-9 in chemoresistance of GyCs

MMP-2 and MMP-9 overexpress in GyCs and promote drug resistance through different mechanisms (Fig. 24.2). MMP-2, a gelatinase A, breaks down various substrates involved in ECM degradation, and its overexpression is linked to aggressiveness as well as malignancy of cancer. MMP-2 has been linked to chemoresistance, through the upregulation of EMT markers. For example, Notch 1 signaling pathway upregulation increases MMP-2, Snail, and vimentin expression, promoting EMT-dependent chemoresistance. The transcription

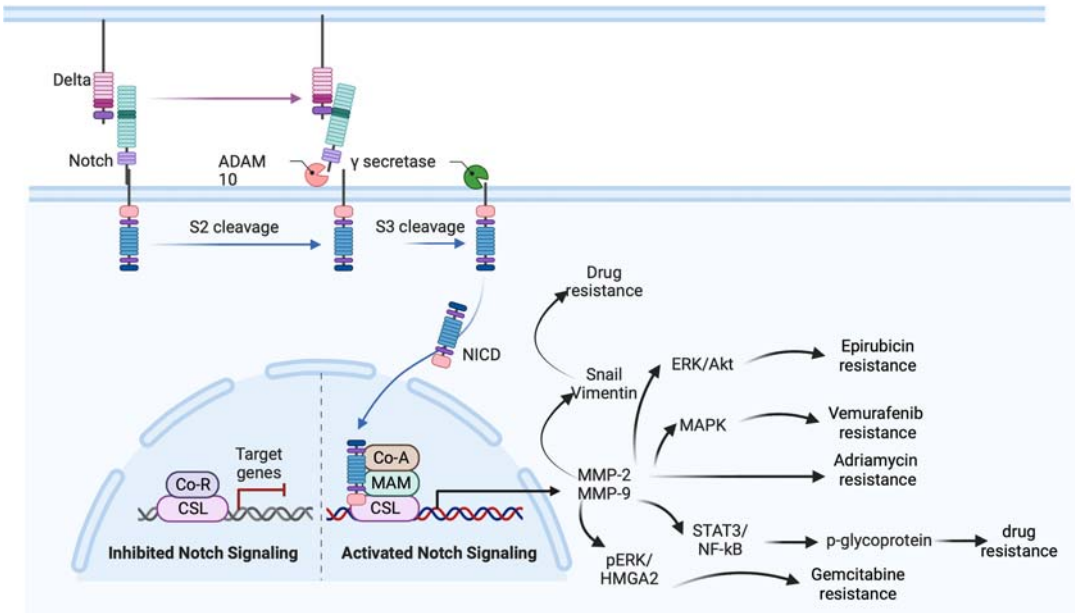

FIGURE 24.2 MMP-2 and MMP-9 mediates drug resistance through different mechanisms. Notch signalling transcriptionally upregulates the expression of MMP-2 and MMP-9. MMP-2 induces drug resistance by enhancing the expression of Snal and vimentin. It also induces resistance to epirubicin via activating ERK/Akt pathway, vemurafenib via activating MAPK signalling, and adriamycin resistance. However, MMP-9 promotes drug resistance by enhancing the expression of p-glycoprotein. It also mediates gemcitabine resistance by phosphorylating ERK and HMGA2 signalling.

factor nuclear factor, erythroid 2-like 2 (NFE2L2), also promotes temozolomide-induced chemoresistance by transcriptionally regulating MMP-2 (Winkler et al., 2020).

MMP-2 and MMP-9 mediate drug resistance via distinct mechanisms. MMP-2 and MMP-9 expression is transcriptionally upregulated by Notch signaling. MMP-2 promotes drug resistance by increasing the expression of Snail and vimentin. It also causes resistance to epirubicin by activating the ERK/Akt pathway, vemurafenib by activating MAPK signaling, and adriamycin resistance. However, MMP-9 enhances drug resistance by increasing the expression of p-glycoprotein. It also promotes gemcitabine resistance by phosphorylating ERK and HMGA2 signaling.

Elevated MMP-2 and MMP-9 induce resistance to epirubicin in OC by elevating orosomucoid 1, activating Akt and ERK signaling pathways. Moreover, MMP-2 activity contributes to vemurafenib resistance, involving MAPK pathway reactivation. PTX-resistant CC cells and adriamycin-resistant osteosarcoma cells also exhibit increased MMP-2 activity, linking MMP-2 to chemoresistance (Qiong and Yin, 2021). Nonetheless, MMP-9, a gelatinase, cleaves various substrates and is extensively studied in cancer metastasis. It promotes invasion by degrading the ECM and inducing EMT. MMP-9 contributes to chemoresistance through the STAT3 and NF-κB signaling pathway. MMP-9 overexpression is related to poor prognosis, and its reduction enhances chemosensitivity. Also, MMP-9 increases P-glycoprotein (Pgp) activity, imparting resistance to therapy. MMP-9 overexpression may lead to chemoresistance by upregulating VEGF, and gemcitabine resistance is obtained by phosphorylating ERK1/2 and increasing HMGA2 expression (Augoff et al., 2022).

The vulnerability of cancer therapy lies in the insidious presence of chemoresistant cancer cells, often rooted in the indefinable CSCs and their proficiency at evading apoptosis. Even a tiny fraction of CSCs within the heterogenous tumor landscape can orchestrate resistance, leading to treatment failure and disease progression. The mechanisms underlying this resilience are as diverse as the cancers (Rezayatmand et al., 2022). A key player is the ABC transporter family, including Pgp, MDR1, and ABCG2, which act as molecular pumps to efficiently expel therapeutic agents from the cell. This "drug efflux" phenomenon is widespread in various resistant cancers, with each transporter exhibiting specificity toward certain drugs. Beyond efflux, genetic and epigenetic alterations can render drugs ineffective by subverting the apoptotic pathway, for instance, TP53 mutation, a common mechanism in cancer, where the normal apoptotic response is hindered, allowing mutated cells to persist. Similarly, gene mutations such as p53 and Bcl2 create aberrant protein functions that impede programmed cell death (Hassin and Oren, 2023).

Metabolic reprogramming also contributes to chemoresistance. Enzymes such as thymidylate synthase can sabotage the action of 5-FU by salvaging its target metabolite, rendering the drug useless. Similarly, glutathione S-transferase enzymes can dismantle drug molecules within cells. These are just glimpses into the vast repertoire of resistance mechanisms, with microRNAs, the TME, and drug-specific vulnerabilities adding further layers of complexity (Kumar et al., 2023). This intricate multidimensionality underscores the immense challenge of overcoming chemoresistance. It necessitates a multifaceted approach that delves deeper into the intricate molecular and environmental cues that empower cancer cells to defy treatment.

3. Strategies to reverse chemoresistance

MMPs levels are low under normal conditions but overexpressed in pathological conditions like cancer. The investigation on downregulation of MMP expression as well as activity as a likely strategy to alleviate chemoresistance has been ongoing. The initial focus on MMP inhibitors (MMPIs), employing zinc ion chelation in their structure, encountered challenges in clinical trials, where side effects offset benefits. This led to further research into alternative approaches, including inhibitory antibodies against MMPs (Mustafa et al., 2022). Antibody inhibitors such as andecaliximab (GS-5745) and DX-2400 have demonstrated promise in early clinical as well as preclinical trials. Synthetic MMPIs, such as a selective inhibitor of MMP-9/MMP-2, have exhibited chemosensitizing effects in vitro, although the precise mechanism of action remains unclear (Shah et al., 2018). Nonanticancer drugs, such as metformin used for type 2 diabetes, have also shown to sensitize tumor to anticancer drugs. It sensitizes by inhibiting the expression of MMP-9 along with sorafenib via targeting specific signaling pathways and reducing cell migration and invasion (Zhang et al., 2023).

In addition to direct MMP targeting, utilizing MMP-responsive nanomaterials has gained attention for enhancing chemotherapeutic agent delivery and anticancer activity. These nanocarriers, activated by MMP activity, ensure precise drug delivery to highly metastatic sites. Various nanocarriers, including macromolecule-based conjugates, liposomes, micelles, and polymeric nanoparticles, have been developed to improve drug penetration, retention, and controlled release in response to MMP activity (Yao et al., 2018).

Understanding promising regulators of MMPs in drug resistance involves exploring tissue inhibitors, microRNAs, and epigenetic regulators. They include DNA methylation and histone modification and may regulate MMPs indirectly, providing alternative strategies for inducing chemosensitivity. Tissue inhibitors, such as α2-macroglobulin and specific inhibitors such as TIMPs, can block MMP activities, and their altered expression levels can impact chemoresistance. miRNAs, such as miR-211, can suppress MMP expression, potentially enhancing drug sensitivity (Cabral-Pacheco et al., 2020).

Extensive research has been conducted on MMPIs in cancer treatment to address chemoresistance, particularly in association with EMT. Earlier MMPIs, such as hydroxamic acid-based batimastat, demonstrated efficacy in preclinical contexts; however, their clinical trials encountered challenges due to broad-spectrum affinity, resulting in diverse side effects. Consequently, an ongoing exploration of alternative MMPIs characterized by more targeted specificity exists. The investigation into therapeutics targeting MMPs in chemoresistance has unveiled a range of inhibitors, encompassing hydroxamic acid-based variants (e.g., batimastat), inhibitory antibodies such as andecaliximab and DX-2400, and synthetic MMPIs such as $C_{21}H_{19}NO_4S$ (Tlatli and El Ayeb, 2013). Despite encouraging outcomes in preclinical trials, the clinical application of these MMPIs faces impediments such as drug resistance and toxicity.

Nonanticancer drugs such as metformin and unconventional approaches, including downregulating MMP activation pathways, exhibit potential chemosensitizing effects. Studies exploring the downregulation of MMPs to enhance cancer cell sensitivity to anticancer drugs have employed diverse strategies, such as using antihypertensive drugs (e.g., enalapril), silencing ETS1 expression, suppressing annexin A5, and utilizing phytochemical compounds

such as cernumidine extract and cordycepin. These approaches aim to counter chemoresistance by indirectly modulating MMP expression (Gavrin and Pyl'tsov, 1966).

In the domain of nanomedicine, the development of MMP-responsive nanomaterials has emerged to augment drug delivery and anticancer activity. Nanocarriers activated by MMP activity facilitate targeted drug delivery to metastatic sites, potentially mitigating drug resistance (Niland et al., 2022). Examples include MMP-2-responsive hyaluronic acid conjugated to poly(amidoamine) carriers and PEG-phosphoethanolamine copolymers reliant on MMP-2 cleavage. These nanomaterials showcase heightened drug delivery efficiency and reduced drug resistance in cancer cells (Xiong and Gao, 2017). Understanding these regulators holds promise for devising novel therapeutic strategies to modulate MMP expression and enhance chemosensitivity (Yao et al., 2018).

Angiogenesis is regulated by VEGF, PEDF, FGF, angiopoietins, and endoglins, while inhibitors include antiangiogenic peptides, hormone metabolites, and apoptosis modulators. The imbalance, termed the angiogenic switch, leads to increased tumor vascularization, promoting tumor progression. AATF, elevated in cancer, interacts with STAT3, upregulating MMP2 and MMP9, deleting the antiangiogenic factor PEDF. Inhibiting AATF reduces MMP2 and MMP9, preserving functional PEDF and inhibiting angiogenesis. This mechanism also involves PEDF blocking VEGFR2 activation, which offers specificity and potential advantages over systemic antiangiogenic compounds with adverse effects (Suresh et al., 2023). Fig. 24.2 depicts the strategies to overcome chemoresistance.

4. Future direction

Future aspects encompass a multifaceted approach to advancing the development of précised and targeted MMP inhibitors (MMPIs) by combining different MMPIs to achieve synergistic effects. They are developing therapies to modulate epigenetic changes to sensitize cancer cells, potentially reversing chemoresistance; furthermore, improve and explore MMP-responsive nanomaterials for enhanced drug delivery; investigate the specific roles of MMPs in the multifaceted landscape of chemoresistance, considering factors such as ABC transporters, genetic alterations, and metabolic reprogramming; and identify novel biomarkers associated with MMP-mediated chemoresistance to guide personalized treatment strategies. They understand the dynamic changes in MMP expression and angiogenesis throughout GyCs.

5. Conclusion

In conclusion, targeting MMPs, mainly through advanced nanomedicine approaches, holds promise for improving the effectiveness of cancer therapies. Exploring the biological regulators of MMPs and their role in neovascularization provides valued insights into emerging novel therapeutic strategies for GyCs. The complexity of chemoresistance calls for a multifaceted approach, exploring various inhibitors and biological regulators to enhance treatment outcomes in gynecological cancers.

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CHAPTER

25

The link between trypsinogen and chymotrypsinogen in gastrointestinal cancer

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1. Introduction

Gastrointestinal (GI) cancers, which include malignancies affecting the esophagus, stomach, small intestine, colon, rectum, pancreas, liver, and gallbladder, represent a significant global health burden. With millions of new cases diagnosed each year and substantial mortality rates, GI cancers remain challenging to manage despite advancements in diagnosis and treatment. Chemotherapy plays a critical role in the treatment paradigm for these cancers (Arnold et al., 2020). However, a major obstacle in the successful treatment of GI cancers is the development of chemoresistance, wherein cancer cells become resistant to the effects of chemotherapy drugs, leading to treatment failure and disease progression. Chemoresistance poses a formidable challenge in the management of GI cancers. Despite initial response to chemotherapy, cancer cells can adapt and develop mechanisms to evade the cytotoxic effects of anticancer drugs. This phenomenon significantly limits the effectiveness of chemotherapy regimens and contributes to treatment failure and disease recurrence. Moreover, chemoresistance can result in the need for more aggressive treatments, increased healthcare costs, and reduced quality of life for patients (Ramos et al., 2021).

Understanding the mechanisms underlying chemoresistance in GI cancers is crucial for developing strategies to overcome this challenge. Research suggests that various factors contribute to chemoresistance, including genetic mutations, altered drug metabolism,

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enhanced DNA repair mechanisms, and changes in cell signaling pathways. Additionally, the tumor microenvironment plays a critical role in mediating chemoresistance by providing a supportive niche for cancer cell survival and proliferation. Addressing chemoresistance requires a multifaceted approach that considers the complex interplay between cancer cells, the tumor microenvironment, and therapeutic agents. Developing targeted therapies that disrupt key pathways involved in chemoresistance, such as DNA repair mechanisms or cell survival signaling pathways, may improve treatment outcomes for patients with GI cancers. Moreover, personalized medicine approaches, including molecular profiling and biomarker identification, hold promise for identifying patients at higher risk of developing chemoresistance and guiding treatment decisions. Overall, advancing our understanding of chemoresistance mechanisms in GI cancers is essential for improving treatment strategies and outcomes in these malignancies (Housman et al., 2014).

2. Proteases and chemoresistance

Proteases are a diverse group of enzymes responsible for catalyzing the hydrolysis of peptide bonds in proteins. They play crucial roles in various physiological processes, including digestion, blood coagulation, wound healing, and immune response. In cancer, proteases are often dysregulated and contribute to tumor progression and metastasis by degrading extracellular matrix components, facilitating angiogenesis, promoting invasion, and modulating signaling pathways involved in cell survival and proliferation. Moreover, emerging evidence suggests that proteases also play a significant role in mediating chemoresistance, posing a challenge to the efficacy of chemotherapy in cancer treatment (Verhamme et al., 2019).

3. Trypsinogen and chymotrypsinogen in GI cancer

Trypsinogen and chymotrypsinogen are precursor enzymes synthesized in the pancreas and released into the duodenum as part of pancreatic juice. Upon activation through proteolytic cleavage, trypsinogen transforms into trypsin, and chymotrypsinogen converts into chymotrypsin. These enzymes play a crucial role in protein digestion in the small intestine. However, in various gastrointestinal (GI) cancers, including pancreatic, colorectal, and gastric cancers, abnormal expression and activation of trypsinogen and chymotrypsinogen have been detected. The dysregulation of trypsinogen and chymotrypsinogen in GI cancers may contribute to tumor development and progression through multiple mechanisms. Firstly, excessive activation of these enzymes can lead to increased proteolytic activity, promoting the degradation of extracellular matrix components and facilitating tumor invasion and metastasis. Moreover, trypsin and chymotrypsin can directly cleave and activate growth factors and cytokines, thereby stimulating cell proliferation and angiogenesis within the tumor microenvironment (Nemoda and Sahin-Tóth, 2006).

Additionally, aberrant expression of trypsinogen and chymotrypsinogen may disrupt normal cellular processes and signaling pathways, leading to uncontrolled growth and survival of cancer cells. For example, activation of trypsinogen and chymotrypsinogen may trigger

downstream signaling pathways involved in cell proliferation, such as the MAPK/ERK and PI3K/Akt pathways, promoting tumor growth and chemoresistance. Furthermore, trypsinogen and chymotrypsinogen may modulate the immune response within the tumor microenvironment, influencing the recruitment and activation of immune cells and promoting immune evasion by cancer cells. This dysregulation of immune signaling may contribute to tumor immune escape and resistance to immunotherapy in GI cancers (Kim et al., 2020). Overall, the aberrant expression and activation of trypsinogen and chymotrypsinogen in GI cancers highlight their potential role as diagnostic and prognostic markers, as well as therapeutic targets. Further research into the mechanisms underlying their dysregulation and their impact on tumor biology is warranted to develop novel treatment strategies for GI cancers.

3.1 Trypsinogen

Trypsinogen stands as a pivotal player in the orchestra of digestive enzymes, representing the inactive precursor to the indispensable trypsin. Synthesized and released by the pancreas, this proenzyme, also known as a zymogen, holds a key position in the intricacies of protein digestion within the gastrointestinal system (Lerch and Gorelick, 2000).

3.2 Molecular genesis

The molecular genesis of trypsinogen constitutes a fascinating exploration into the intricate design and functionality of this proenzyme. Synthesized within the exocrine cells of the pancreas, trypsinogen serves as the inactive precursor to the crucial digestive enzyme trypsin. This discourse delves into the structural intricacies and regulatory mechanisms that govern the molecular birth of trypsinogen (González-Titos et al., 2021).

3.2.1 Primary structure

At the heart of trypsinogen's molecular genesis lies its primary structure, characterized by a single polypeptide chain. This chain is intricately folded, creating a specific three-dimensional conformation that sets the stage for the subsequent activation processes. The molecular intricacies embedded within this structure lay the foundation for trypsinogen's role in the digestive cascade (Leboffe et al., 2015).

3.2.2 Catalytic triad and activation sites

Central to trypsinogen's molecular blueprint is the presence of the catalytic triad, a trio of amino acid residues - histidine, aspartic acid, and serine. This triad, strategically positioned within the polypeptide chain, serves as the catalytic machinery essential for the enzymatic activity of trypsin. Additionally, trypsinogen harbors specific activation sites that render it susceptible to cleavage and transformation into its active counterpart (Cai et al., 2022).

3.2.3 Synthesis and folding

The synthesis of trypsinogen occurs within the endoplasmic reticulum of the pancreatic exocrine cells. During this process, the linear sequence of amino acids is meticulously assembled, followed by intricate folding facilitated by chaperone proteins. This folding is crucial for

establishing the correct spatial arrangement of the catalytic triad and activation sites, ensuring the latent functionality of trypsinogen (Leboffe et al., 2015).

3.2.4 Inhibition mechanisms

To prevent premature activation within the pancreas, trypsinogen is equipped with specific inhibitory mechanisms. These mechanisms act as molecular safeguards, inhibiting the premature activation of trypsinogen in the pancreatic environment. This inhibition ensures that the transformative process into trypsin occurs at the precise moment in the small intestine, where protein digestion is intended to take place (Leboffe et al., 2015; Cai et al., 2022).

3.2.5 Transport, release and activation cascade

The life cycle of trypsinogen, the inert precursor to the powerhouse digestive enzyme trypsin, is a ballet of intricate movements orchestrated within the pancreas and small intestine. From synthesis to activation, each step in this journey is meticulously designed to ensure precise functionality and prevent untimely activation, safeguarding the delicate balance of digestive processes. Trypsinogen's journey commences within the exocrine cells of the pancreas, where it is synthesized as a single polypeptide chain. This initial structural blueprint sets the stage for subsequent events. As the nascent polypeptide chain emerges, chaperone proteins guide its intricate folding within the endoplasmic reticulum. This carefully orchestrated folding is not merely a formality; it dictates the spatial arrangement of critical elements, such as the catalytic triad, crucial for enzymatic activity. After the synthesis and folding duet, trypsinogen is enveloped into vesicles and embarks on a journey within the pancreatic cell. These vesicles act as protective capsules, shielding trypsinogen from the harsh pancreatic environment. The transportation choreography ensures the safe passage of this enzyme precursor to the next stage of its life cycle. The carefully choreographed movements continue as the vesicles release their cargo into the pancreatic duct system. This controlled release mechanism is not arbitrary; it serves a crucial purpose—to prevent the premature activation of trypsinogen within the pancreas itself. Any untimely activation could result in self-digestion, compromising the integrity of pancreatic tissues. Thus, the release into the duct system is akin to a controlled descent, guiding trypsinogen toward its next destination. The journey of trypsinogen takes an intermission as it awaits its cue in the small intestine, specifically the duodenum. This is where the digestive drama unfolds with the entrance of the enzyme enteropeptidase, the catalyst that will propel trypsinogen into the limelight (Gerasimenko et al., 2011; Pallagi et al., 2020).

Upon reaching the duodenum, trypsinogen encounters enteropeptidase, a key player in the activation cascade. This enzyme acts as a director, cueing the precise moment for trypsinogen's transformation. The activation cascade is initiated, setting the stage for trypsinogen's metamorphosis into the active enzyme trypsin. Enteropeptidase' role is akin to a choreographer, orchestrating the activation cascade with finesse. This cascade involves specific cleavage of trypsinogen at activation sites, unraveling its latent catalytic potential. The transformation into trypsin is not a sudden eruption but a calculated sequence, a molecular ballet where each step is executed with precision. In essence, the journey of trypsinogen, from its synthesis and folding to the activation cascade, is guided by a meticulously designed molecular blueprint. This blueprint encapsulates the primary structure, catalytic triad, synthesis-folding duo, inhibition mechanisms, and the activation cascade. It is a symphony of molecular elements

working in harmony, revealing the precision and complexity embedded within the functionality of trypsinogen. This intricately woven molecular ballet has broader implications for the gastrointestinal symphony. Research by Gerasimenko et al. (2011) and Pallagi et al. (2020) has delved into these intricacies, shedding light on the molecular nuances that govern trypsinogen's journey. Understanding this symphony contributes to our comprehension of effective protein digestion within the gastrointestinal system, offering insights into maintaining digestive harmony. Trypsinogen's journey is not merely a biochemical process; it is a ballet of molecular intricacies. From its inception in the pancreas to the grand finale in the small intestine, trypsinogen's journey exemplifies the precision and coordination embedded within the digestive symphony, contributing to our understanding of gastrointestinal health.

4. Catalytic proficiency of trypsinogen

The catalytic proficiency of trypsinogen serves as a cornerstone in the digestive cascade, where this proenzyme undergoes a transformative process to become the potent digestive enzyme trypsin. Delving into the catalytic mechanisms, activation sites, and enzymatic activities of trypsinogen provides insights into its pivotal role in protein digestion within the gastrointestinal system. Central to trypsinogen's catalytic proficiency is the catalytic triad, composed of histidine, aspartic acid, and serine residues. This triad forms the molecular machinery responsible for the enzymatic activity of trypsin. Positioned strategically within the polypeptide chain, these residues are primed for action upon activation, facilitating the hydrolysis of peptide bonds in proteins.

Trypsinogen's catalytic proficiency is underscored by its **activation cascade and structural transformation, enzymatic substrate specificity, kinetics of catalysis, pH dependence, and optimal conditions and regulatory mechanisms.** It is unleashed through a carefully orchestrated activation cascade. Upon encountering the enzyme enteropeptidase in the small intestine, trypsinogen undergoes site-specific cleavage, leading to a structural transformation. This transformation exposes the catalytic triad and converts trypsinogen into trypsin, unlocking its enzymatic potential. It is also underscored by its exquisite substrate specificity. As a serine protease, trypsin selectively cleaves peptide bonds adjacent to specific amino acids, namely lysine and arginine. This specificity ensures targeted hydrolysis of proteins into smaller peptides and amino acids, facilitating optimal nutrient absorption in the digestive process. It is further exemplified by its enzymatic kinetics. Trypsin exhibits Michaelis–Menten kinetics, wherein the rate of enzymatic reaction is dependent on the substrate concentration. This characteristic ensures that trypsin operates efficiently in the dynamic and variable environment of the digestive system (Rall and Bordusa, 2002; Zou et al., 2022).

Additionally, it is intricately tied to its pH dependence. Trypsin operates optimally in a slightly alkaline environment, such as the pH range found in the small intestine. This pH dependence ensures that trypsinogen is activated and catalytically active in the specific anatomical compartments where protein digestion predominantly occurs. The catalytic proficiency of trypsinogen is also subject to stringent regulatory mechanisms. Inhibitors, such as pancreatic trypsin inhibitor, act as molecular safeguards, preventing excessive enzymatic activity and maintaining the delicate balance of the digestive process. This regulatory

framework ensures controlled protein digestion without compromising the integrity of surrounding tissues (Rall and Bordusa, 2002).

5. Clinical implications

The clinical implications of trypsinogen extend beyond its role as a precursor to trypsin in the digestive cascade. Dysregulation in its activation or enzymatic activity holds significant repercussions, shedding light on its clinical relevance in various gastrointestinal conditions. This exploration delves into the clinical implications of trypsinogen dysfunction, particularly in the context of pancreatic disorders.

5.1 Pancreatitis: A consequence of dysregulation

One of the primary clinical implications of trypsinogen lies in its association with pancreatitis. Aberrant activation or premature activation of trypsinogen within the pancreas can lead to inflammation and tissue damage, initiating a cascade of events characteristic of pancreatitis. This inflammatory condition, marked by abdominal pain, digestive disturbances, and potential systemic complications, underscores the clinical significance of maintaining the precise regulation of trypsinogen (Bush and Akshintala, 2023).

5.2 Trypsinogen dysfunction in pancreatic disorders

Beyond pancreatitis, trypsinogen dysfunction has been implicated in various pancreatic disorders. Genetic mutations affecting trypsinogen or its regulatory mechanisms may contribute to the pathogenesis of conditions such as hereditary pancreatitis. Understanding the molecular underpinnings of trypsinogen-related disorders is crucial for advancing diagnostic and therapeutic approaches (Derikx and Drenth, 2010).

5.3 Diagnostic biomarker in gastrointestinal cancers

Recent research has uncovered a potential link between trypsinogen and gastrointestinal cancers. Elevated levels of trypsinogen or aberrant activation pathways have been identified in certain cancer types, including pancreatic and colorectal cancers. This revelation opens avenues for exploring trypsinogen as a diagnostic biomarker, offering insights into early cancer detection and prognosis (Masson et al., 2023).

6. Therapeutic targets

The clinical implications of trypsinogen extend to therapeutic considerations. Targeting trypsinogen activation pathways or modulating its enzymatic activity represents a promising avenue for developing treatments for pancreatic disorders. Small molecule inhibitors and other interventions aimed at restoring the balance of trypsinogen in disease states are

currently under investigation. Advances in genomic profiling have unraveled specific mutations associated with trypsinogen dysfunction. This knowledge paves the way for precision medicine approaches, wherein therapies can be tailored based on the underlying molecular alterations in individual patients. Understanding the genetic landscape of trypsinogen-related disorders enhances the potential for personalized and targeted interventions. The clinical implications of trypsinogen are continually evolving with ongoing research. Future directions include expanding our understanding of trypsinogen's role in gastrointestinal cancers, identifying additional therapeutic targets, and refining diagnostic strategies. The integration of cutting-edge technologies and a deeper molecular understanding of trypsinogen hold the promise of advancing clinical care in the realm of digestive health (Masson et al., 2023). The clinical implications of trypsinogen span a spectrum of gastrointestinal conditions, from pancreatitis to cancer. Unraveling the threads of its dysfunction opens avenues for diagnostic innovation, therapeutic interventions, and the potential for personalized medicine. As research progresses, the clinical landscape of trypsinogen-related disorders is poised for transformative advancements, offering new hope for patients and clinicians in the realm of digestive health.

6.1 Chymotrypsinogen

Chymotrypsinogen, a crucial precursor orchestrating the intricate digestive cascade, assumes a central role not only in dietary protein breakdown but also in the context of gastrointestinal (GI) cancers. As a proenzyme originating from the pancreas, chymotrypsinogen maintains an inactive state until meticulously activated in the duodenum, a process orchestrated by the enzyme trypsin. This activation transforms it into the active form known as chymotrypsin, highlighting its role in the digestive process (Szmola and Sahin-Tóth, 2010). The structural intricacies of chymotrypsinogen, characterized by distinct regions including the catalytic segment, unveil its unique role in targeting specific peptide bonds adjacent to aromatic amino acids during protein digestion. Beyond its structural and activation nuances, this exploration delves into the physiological significance of chymotrypsinogen, especially in the context of GI cancers. Collaborating with other pancreatic enzymes, it contributes to the efficient breakdown of complex proteins into absorbable components, supporting the body's nutritional needs. The regulatory mechanisms governing the release and activation of chymotrypsinogen not only showcase the body's precision in enzyme control but also play a crucial role in preventing untimely activation and potential self-digestion of pancreatic tissues (Shaw et al., 2007). This comprehensive examination illuminates the fundamental aspects of chymotrypsinogen, setting the stage for understanding its clinical implications in the intricate landscape of GI cancers. Disorders stemming from dysregulation or premature activation of chymotrypsinogen underscore its importance in the realm of GI cancers. Ultimately, chymotrypsinogen assumes a multifaceted role in the intricate realm of digestive physiology, weaving together precision, regulation, and functional significance in the intricate orchestration of nutrient assimilation. Moreover, its potential implications in the development of gastrointestinal (GI) cancers add a layer of complexity to its significance in physiological processes (Nemoda and Sahin-Tóth, 2006).

6.1.1 Structure of chymotrypsinogen

Chymotrypsinogen, identified as a serine protease, plays a crucial role as a proenzyme or zymogen designed to maintain an inactive state until it reaches its designated site of action. Originating from the pancreas, its primary function is to prevent autodigestion of pancreatic tissues, emphasizing its pivotal role in maintaining the integrity of this vital organ. Structurally, chymotrypsinogen consists of a single polypeptide chain, spanning approximately 245 amino acids. This chain exhibits distinct segments, including the N-terminal, catalytic, and C-terminal regions. The N-terminal region often plays a role in facilitating the activation process, marking a critical step in transforming the proenzyme into its active form. The catalytic region, situated within the chain, harbors the active site responsible for enzymatic activity (Szmola and Sahin-Tóth, 2010). However, in its inactive zymogen state, this catalytic region remains obstructed, preventing premature enzymatic activity. Released in an inactive form, chymotrypsinogen undergoes activation through a series of events, typically involving the cleavage of specific peptide bonds. This activation mechanism is crucial for regulating the enzyme's activity and preventing unwanted proteolysis. Understanding the structural intricacies of chymotrypsinogen sheds light on the sophisticated regulatory mechanisms that govern its function and underscores its significance in maintaining physiological balance within the digestive system.

6.1.2 Activation of chymotrypsinogen

Chymotrypsinogen undergoes activation in the duodenum, a critical phase in its functional journey. Activation is facilitated by the encounter with trypsin, an enzyme specifically designed for this purpose. In this orchestrated molecular dance, trypsin cleaves a particular peptide bond within the chymotrypsinogen structure, leading to the removal of an inhibitory peptide fragment. This strategic cleavage event marks the metamorphosis of chymotrypsinogen into its active and potent form chymotrypsin. The precision of this activation mechanism is paramount. By ensuring specificity in the removal of the inhibitory fragment, chymotrypsin becomes active exclusively at the site of protein digestion in the small intestine. This specificity not only highlights the intricacies of the digestive process but also underscores the importance of tightly regulated enzymatic activation in maintaining the overall efficiency of the digestive system (Nemoda and Sahin-Tóth, 2006). Comprehending the carefully coordinated activation of chymotrypsinogen offers valuable insights into the intricate molecular dance that governs digestive processes. This highlights the nuanced equilibrium essential for optimal physiological functioning, showcasing the complex interplay of enzymes within the gastrointestinal tract.

6.1.3 Function and regulation of chymotrypsin activity

Chymotrypsin plays a vital role in the digestive process, breaking down dietary proteins into smaller peptides. Its substrate specificity lies in cleaving peptide bonds adjacent to aromatic amino acids such as phenylalanine, tryptophan, and tyrosine. This specificity allows chymotrypsin to target specific sites on protein molecules, facilitating the efficient digestion of diverse dietary proteins. To prevent premature activation and potential harm to pancreatic tissues, chymotrypsin activity is tightly regulated. The release of chymotrypsinogen into the small intestine is controlled by hormonal signals stimulated primarily by the presence of food

(Ma et al., 2005). Furthermore, the conversion of chymotrypsinogen to chymotrypsin is regulated by trypsin activity, ensuring a controlled and stepwise activation process.

6.1.4 Physiological significance

Chymotrypsinogen and its active form, chymotrypsin, are integral components of the digestive system. They collaborate with other pancreatic enzymes to break down ingested proteins into peptides and amino acids, which the body absorbs and utilizes for various physiological functions. The regulated release and activation of chymotrypsinogen exemplify the complexities of digestive enzyme control mechanisms in maintaining homeostasis (Zhou and Sahin-Tóth, 2011).

6.1.5 Clinical implications

Clinical implications associated with disturbances in the regulation or activation of chymotrypsinogen are paramount, as they can precipitate pancreatic disorders. Premature activation of chymotrypsinogen within the pancreas poses a significant risk, potentially leading to autodigestion and pancreatitis. The intricate interplay of chymotrypsinogen and related enzymes is a focal point for researchers and clinicians. By delving into the complexities of these enzymatic processes, they aim to unravel the underlying mechanisms of pancreatic diseases. This in-depth understanding becomes the foundation for developing targeted therapeutic interventions aimed at mitigating the impact of disruptions in chymotrypsinogen regulation. The quest to decipher the intricacies of chymotrypsinogen's role in pancreatic disorders is crucial for advancing medical knowledge and improving clinical outcomes (Nemoda and Sahin-Tóth, 2006). As researchers continue to unravel the mysteries surrounding chymotrypsinogen, the potential for innovative treatments and interventions in the realm of pancreatic diseases emerges, promising new avenues for enhancing patient care and management strategies.

6.1.6 In-depth exploration of chymotrypsinogen

Chymotrypsinogen, as the precursor to the active enzyme chymotrypsin, holds a pivotal position in the digestive cascade. Its structural intricacies provide a foundation for understanding its activation and subsequent functional role. The initiation of the activation process occurs in the duodenum, where trypsin acts as a key player. This step is a protective mechanism, ensuring that chymotrypsin becomes active only in the specific environment of the small intestine. The functional significance of chymotrypsin lies in its ability to cleave peptide bonds adjacent to aromatic amino acids (Szabó et al., 2015). This unique substrate specificity allows it to play a key role in the digestion of a variety of dietary proteins. The precision with which it targets specific sites on protein molecules showcases its efficiency in breaking down complex structures into more manageable components. The regulation of chymotrypsinogen activity is a finely tuned process. Hormonal signals triggered by the presence of food control its release into the small intestine. Once released, the conversion from chymotrypsinogen to chymotrypsin is orchestrated by trypsin, ensuring a controlled and gradual activation. This regulatory mechanism prevents untimely enzyme activity, safeguarding the pancreas from self-digestion. In terms of physiological significance, chymotrypsinogen is not merely a digestive enzyme but a key player in the larger context of nutrient absorption and utilization. Its collaboration with other pancreatic enzymes

contributes to the breakdown of dietary proteins into smaller, absorbable components. This process is essential for meeting the body's nutritional requirements and maintaining overall health. On a clinical front, disturbances in the regulation or activation of chymotrypsinogen can have significant repercussions (Fu et al., 2021). Pancreatitis, characterized by inflammation of the pancreas, may occur if chymotrypsinogen is prematurely activated within the pancreatic tissues. Understanding the nuances of chymotrypsinogen regulation becomes crucial in the diagnosis and management of such pancreatic disorders. The ongoing research on chymotrypsinogen and related enzymes aims to unravel deeper insights into pancreatic diseases. Scientists and clinicians are continually exploring potential therapeutic interventions that could modulate chymotrypsinogen activity, offering novel approaches for treating pancreatic disorders (Szmola and Sahin-Tóth, 2010).

7. Role of chymotrypsinogen in GI cancers

Chymotrypsinogen, primarily recognized for its role in digestion, has garnered increasing attention in the context of gastrointestinal (GI) cancers. Emerging research indicates that the altered expression and activity of chymotrypsinogen may play a significant role in the pathogenesis and progression of GI malignancies. The GI tract encompasses various organs, including the esophagus, stomach, small intestine, and colon, where chymotrypsinogen contributes to the breakdown of proteins during digestion. In GI cancers, aberrations in chymotrypsinogen expression levels have been noted, prompting investigations into its potential implications. Studies suggest that dysregulation of chymotrypsinogen may influence tumor behavior and the microenvironment. Altered enzymatic activity could potentially contribute to the breakdown of extracellular matrix proteins, facilitating tumor invasion and metastasis. Moreover, the interactions between chymotrypsinogen and inflammatory processes within the GI tract may further influence cancer development (Goettig et al., 2019). The exploration of chymotrypsinogen's involvement in GI cancers extends beyond its digestive role. It is becoming increasingly evident that this enzyme might participate in signaling pathways associated with cancer progression. Researchers are unraveling the intricate molecular mechanisms through which chymotrypsinogen may impact cell proliferation, apoptosis, and angiogenesis in the GI context. Understanding the role of chymotrypsinogen in GI cancer has implications for diagnostic and therapeutic strategies. Detection of altered chymotrypsinogen expression patterns could serve as a potential biomarker for certain GI malignancies, aiding in early diagnosis and risk stratification. Targeting chymotrypsinogen-related pathways might offer innovative therapeutic approaches, potentially influencing tumor growth and enhancing treatment outcomes (Lyu et al., 2024).

8. Conclusion

In conclusion, proteases, including trypsinogen and chymotrypsinogen, play multifaceted roles in GI cancer progression and chemoresistance. Understanding the mechanisms by

which these proteases contribute to chemoresistance is crucial for the development of effective therapeutic strategies to overcome this challenge. Targeting proteases or their downstream signaling pathways may offer promising avenues for enhancing the efficacy of chemotherapy and improving outcomes for patients with GI cancer. Further research is needed to elucidate the specific roles of trypsinogen and chymotrypsinogen in mediating chemoresistance and to identify novel targets for therapeutic intervention in GI cancer.

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CHAPTER

26

Role of proteasome in the development of chemoresistance in multiple myeloma

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1. Introduction

"Cancer" describes a broad category of disorders caused by unchecked cell survival, development, and division capable of causing local invasion and/or remote metastases. Cells play an important role in cancer development, which is fundamentally a hereditary disease (Dontu and Wicha, 2005). Every region of the body is susceptible to cancer, which can appear in a variety of ways. It is often divided into four categories: malignancies of the central nervous system, lymphoma and leukemia, sarcoma, and carcinoma (Blackadar, 2016). The incidence of cancer is on the rise in economically growing nations because of demographic changes, physical inactivity, smoking, and Westernized diets, among other factors (Jemal et al., 2011).

Multiple myeloma (MM) is the second most frequent hematological cancer. Because of its rising global frequency, it is an important subject for clinical research (Kazandjian, 2016). MM appears within the complex structure of the bone marrow and blood, and this unrelenting competitor arises from the malignant transformation of plasma cells, an essential part of our immune system, which results in an illness characterized by abnormal proliferation and compromised function (Fairfield et al., 2016). MM, a challenging and untreatable type of cancer, is characterized by the accumulation of abnormal plasma cells in the bone marrow, posing substantial difficulties for patients (Solimando et al., 2020). Understanding the basic

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biology of plasma cells and their function in the immune system is crucial to comprehending the complexities of MM. The production of antibodies by plasma cells, often referred to as effector B cells, is necessary for the body's defense against infections and illnesses (Nagasawa, 2006). Nevertheless, these cells lose their regulatory mechanisms and proliferate uncontrollably when they mutate and are malignant, mostly developing tumors inside the bone marrow. The characteristics of MM, such as anemia, impaired immunological responses, and bone abnormalities, are caused by these tumors that prevent the body from producing healthy blood cells (Al-Farsi, 2013). A major therapeutic difficulty in the treatment of MM is chemoresistance, which can be overcome with the help of proteasome inhibitors. As the proteasome inhibitors block the DNA repair mechanism and encourage the buildup of DNA damage, they make MM cells more susceptible to chemotherapy (Weir et al., 2023).

2. Proteasome

The proteasome is a complex and dynamic protein complex that plays a vital role in eukaryotic cells' regulatory mechanisms, protein breakdown, and cellular homeostasis. It serves as the "recycling center" of the cell, disassembling undesirable or damaged proteins into smaller peptides that are then used for different purposes throughout the cell (Roos-Mattjus and Sistonen, 2004). A coordinated ensemble of proteins, which encompasses the E1, E2, and E3 acting as ubiquitin-ligating enzymes, collaborates to instigate the proteasome's action. The proteasome, a significant energy-consuming protein complex, plays a crucial role in breaking down intracellular proteins. One important molecule that is known to cooperate with the proteasome is the polymerization of ubiquitin, which functions as a degradation signal for many target proteins (Türker et al., 2021). Target proteins are transported by the polymerized ubiquitin chain to the proteasome, where the substrate is hydrolyzed by proteases (Ventii and Wilkinson, 2008).

2.1 Structure of proteasome

In eukaryotic cells, the proteasome is a vast and intricate apparatus for breaking down proteins. It is made up of many subunits arranged into a precise structure that facilitates the effective recycling and breakdown of proteins. The 20S core particle and the regulatory particles attach to either or both of the core particle's ends and exhibit the main structural elements of the proteasome (Tanaka, 2009). The 20S core particle is the central cylindrical structure of the proteasome. The CP or 20S proteasome, also known as the catalytic core particle, is made up of four heptameric rings. Each ring has seven identical α and β structural subunits (Groll et al., 1997). The proteasome belongs to the threonine protease family because the catalytic activity of the β subunits relies on threonine residues at their N-termini. Access to the central proteasome is primarily regulated by the hydrophobic N-terminal tails of the 20Sα subunits, which extend into the interior and serve as a checkpoint to prevent indiscriminate degradation (Bajorek et al., 2003). A major contributor in ubiquitin-dependent protein degradation, the 19S RP of proteasome connects with one or both ends of the 20S catalytic CP. Nine subunits (Rpns 3, 5, 6, 7, 8, 9, 11, 12, and 15) make up the lid subcomplex, and they are essential for deubiquitylation as well as the structural integrity of the proteasome. Of them, Rpn11

functions as the intrinsic deubiquitinase (DUB), cleaving the ubiquitin chain from the substrate using zinc-dependent metalloprotease activity (Huang et al., 2016). The base subcomplex consists of 10 subunits, including four non-ATPase regulatory subunits (Rpns 1, 2, 10, 13) and six AAA+ ATPases (Rpt1-6). Positioned at the interface of the base and lid, the non-ATPase regulatory subunits have a critical function in recognizing and binding ubiquitin (Elsasser et al., 2004). The motor-like properties of the AAA+ ATPase subunits facilitate the transfer of the unfolded polypeptide into the catalytic chamber, which is necessary for substrate unfolding (Bard et al., 2018). Simultaneously, the AAA+ ATPases interact with the 20S CPα subunits through a C-terminal hydrophobic-tyrosine-X motif. This interaction triggers a structural alteration in the N-termini of the α-subunits, which in turn releases the gate and facilitates the movement of unfolded proteins (Yu et al., 2010). These caps stand for unique regulatory complexes that affect the way proteasomes operate. In particular, PA28α and PA28β create heteroheptameric complexes that are mainly present in the cytoplasm (Huber and Groll, 2017), but PA28γ only forms homoheptameric complexes that are found in the nucleus. Functionally, PA28αβ activates the proteasome without the need for ATP or ubiquitin, which has a substantial effect on immunoproteasome function. The proteasome's functional diversity and adaptability are expanded by these different regulatory connections, which enable the sophisticated regulation of particular cellular settings and processes (Dubiel et al., 1992).

2.2 Functions of proteasome

The proteasome performs the following different roles in normal cells.

2.2.1 Protein recycling, degradation, and regulation

The proteasome is the main piece of biological equipment that breaks down and degrades undesirable or damaged proteins. To preserve ideal quantities of protein within cells and their functionality, it targets particular proteins that have been flagged for breakdown, such as misfolded or excess proteins (Thibaudeau and Smith, 2019).

2.2.2 Cell cycle control

Two essential regulatory proteins that govern the various stages of the cell cycle are cyclins and CDKs (Giacinti and Giordano, 2006). The control of cell cycle checkpoints is another function of the proteasome. Checkpoint regulatory proteins are broken down by the proteasome, which either permits the cell cycle to continue or stops it if mistakes are found (Molinari, 2000). Cyclin-CDK complex levels are controlled by proteasomal degradation during various phases of the cell cycle. The proteasome regulates the amounts of pro- and antiapoptotic proteins that affect apoptosis during the cell cycle. The proper balance between apoptosis and cell cycle advancement is maintained by this control (Xu et al., 2008).

2.2.3 Antigen processing and immune response

For cells to process antigens, the proteasome plays a crucial role. It degrades foreign proteins into smaller peptides, a portion of which is displayed by MHC molecules on the cell surface (Sijts and Kloetzel, 2011). The proteasome frequently uses the ubiquitin-proteasome pathway to facilitate the breakdown of proteins. A tiny molecule called ubiquitin marks particular proteins, including antigens, for destruction (Çetin et al., 2021).

2.2.4 Regulation of transcription factors

Transcription factors attach to specific DNA sequences exerting an influential role in governing gene expression through the regulation of gene transcription. Protease-mediated regulation of transcription factors is an essential mechanism that aids in the regulation of gene expression and, by extension, several cellular activities (Filtz et al., 2014). It ensures strict control over gene expression by assisting in the prevention of an excess of activated transcription factors (Geng et al., 2012).

2.3 The action of the mechanism of proteasome in normal cells

2.3.1 NF-κB signaling pathway

A vital signaling cascade involved in many physiological functions, such as immune response, inflammation, cell proliferation, apoptosis, and differentiation, is the NF-κB (nuclear factor-kappa B) pathway. The activation and degradation of NF-κB, vital for the functioning of this pathway, are primarily controlled by the proteasome. The key function of the NF-κB transcription factor family is to control the expression of certain genes (Gambhir et al., 2015). When NF-κB is not active, it is usually bound to inhibitory proteins called IκBs (inhibitors of κB) and sequestered in the cytoplasm. The p50 and p65 (RelA) subunits of NF-κB form a heterodimer, which is the main form of the protein. Numerous stimuli, including proinflammatory cytokines such as TNF-α, and IL-1β, microbial products such as lipopolysaccharides, and stress signals, can activate the NF-κB pathway (Oeckinghaus and Ghosh, 2009). These stimuli start a signaling cascade that eventually causes IκBs to get phosphorylated and then degrade. An enzyme known as IκB kinase (IKK) complex phosphorylates IκB, which is a crucial step in NF-κB activation. IκB becomes polyubiquitinated upon phosphorylation, designating it for proteasome destruction. The swift degradation of IκB, facilitated by the ubiquitin-proteasome system, enables unrestricted translocation of NF-κB into the nucleus, which is depicted in Fig. 26.1. When NF-κB attaches to particular DNA sequences in target gene promoters, known as κB sites, it can activate or repress gene expression within the nucleus (Hoesel and Schmid, 2013). Target genes controlled by NF-κB include those involved in immunological responses, cell survival (Bcl-2, cIAPs), and inflammation. Tight control and prompt termination of NF-κB signaling depend on the proteasome's participation in the NF-κB pathway. The cessation of NF-κB-mediated transcriptional responses can occur when NF-κB is polyubiquitinated and targeted for proteasomal destruction, following the completion of its transcriptional activity (Park and Hong, 2016).

This pathway involves the activation of the receptor by numerous signals followed by the phosphorylation of IκB. The phosphorylated IκB is subjected to ubiquitination and eventually leads to the degradation of IκB and translocation of NF-κB into the nucleus, which plays a vital role in immunological responses.

2.3.2 Ubiquitin-proteasome pathway

The ubiquitin-proteasome system (UPS) is a crucial physiological process that governs the breakdown of particular proteins. It is also essential for preserving cellular homeostasis, directing the progression of the cell cycle, and coordinating many signaling pathways. This mechanism entails binding a little protein called ubiquitin to proteins to flag them for

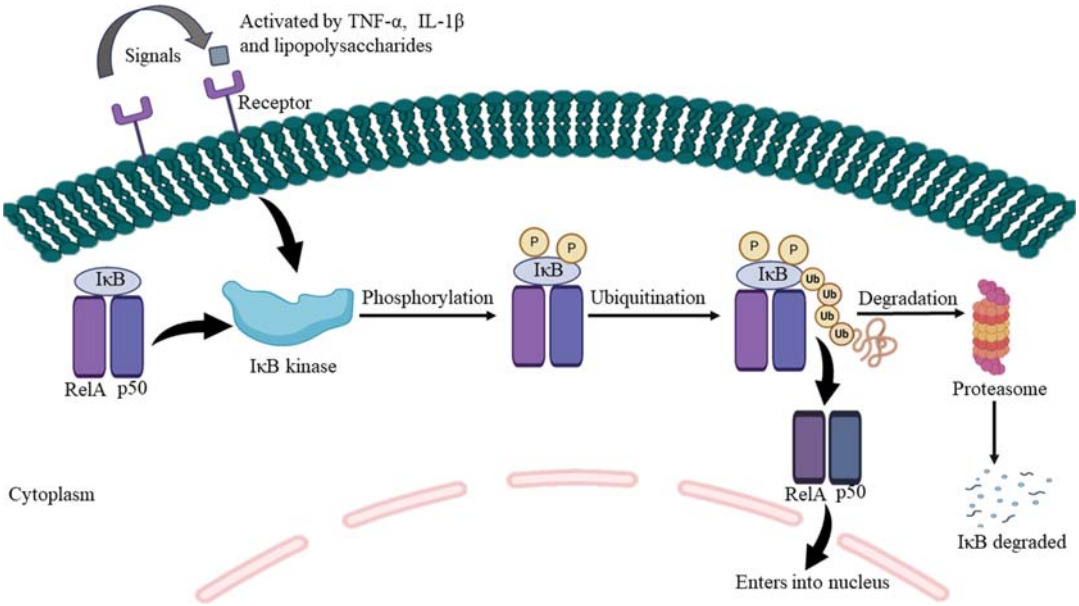

FIGURE 26.1 Mechanism involved in NF-κB signaling pathway.

degradation, which is then followed by proteolytic degradation within the proteasome (Ciechanover and Schwartz, 1998). Target proteins are marked for breakdown by the molecular label ubiquitin, a 76-amino acid protein with a high degree of conservation. An enzyme known as E1 (ubiquitin-activating enzyme) initiates the process by activating ubiquitin. E1 initiates the activation of ubiquitin through an ATP-dependent process, forming a high-energy thioester bond between the C-terminal glycine of ubiquitin and a cysteine residue on E1 (Mani and Gelmann, 2005). The activated ubiquitin molecule has been enzymatically linked to an E2 enzyme. E2 works in conjunction with a ubiquitin ligase (E3) to accurately attach and transfer ubiquitin to a lysine residue on the target protein. As a substrate receptor, the E3 ligase identifies particular target proteins by analyzing their structures or sequences. The E3 ligase aids in the transfer of ubiquitin from the E2 enzyme to the target protein. When multiple ubiquitin molecules are affixed to the target protein, this process is termed polyubiquitination. The polyubiquitin chain acts as a recognition signal for the proteasome, guiding the target protein to the proteasome for degradation (Callis, 2014). A sizable protein complex called the proteasome carries out the protein degradation. It is made up of regulatory particles and the 20S proteasome, which serves as the core particle. Numerous proteolytic subunits with chymotrypsin-, trypsin-, and peptidyl glutamyl-peptide hydrolyzing activity are present in the core particle. The 19S or 11S complexes, which are regulatory particles, are responsible for identifying and attaching to ubiquitinated proteins, unfolding them, and transferring them to the catalytic core where they will be degraded. The target protein is deubiquitinated before it enters the proteasome and is subsequently unfolded, at which point the proteolytic subunits in the proteasome core cut it into tiny peptides. These peptides undergo additional processing to produce amino acids, which are then recycled for the creation

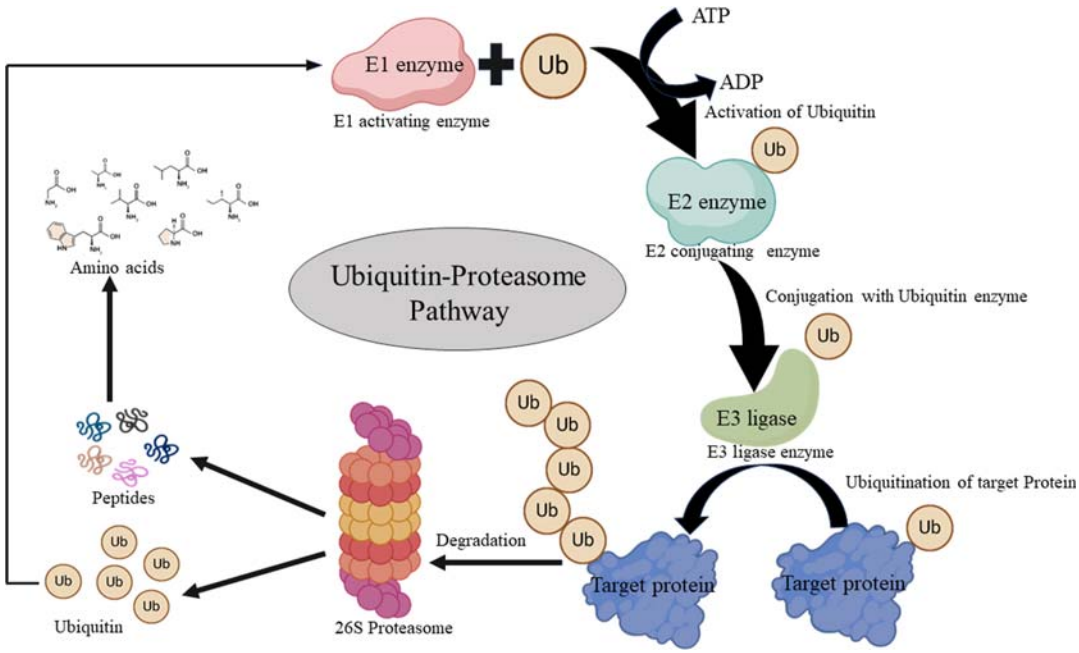

FIGURE 26.2 Mechanisms involved in ubiquitin-proteasome pathway.

of new proteins. This overall mechanism is depicted in Fig. 26.2. By specifically breaking down misfolded, broken, or superfluous proteins, the UPS helps to preserve protein homeostasis by limiting the buildup of harmful protein aggregates inside cells. Numerous illnesses, such as malignancies, autoimmune diseases, and neurodegenerative disorders, are linked to the dysregulation of this system (Tanaka, 2009).

The first step in the pathway is the activation of E1 by ubiquitin followed by the conjugation and ligation of ubiquitin to the target protein with the help of E2 and E3 enzymes. The proteasome causes the degradation of ubiquitinated protein, leading to the breakdown of proteins into peptides.

3. Proteasome and multiple myeloma

The malignant condition known as MM is caused by neoplastic plasma cells that have a strong affinity for bone marrow (BM). Monoclonal immunoglobulins in the urine or blood serve as the hallmark feature of this diseases. The CRAB criteria—renal failure, anemia, hypercalcemia, and bone lesions also known as end-organ damage—are commonly associated with the condition. Characteristics of MM include the presence of M-protein in urine or serum, monoclonal plasma cells present in the BM exceeding 10%, and end-organ destruction (Maes et al., 2013). Generally, monoclonal gammopathy of unknown significance (MGUS), an asymptomatic plasma cell dyscrasia, is the precursor to MM (Landgren et al., 2009). Due to their heightened rates of cell proliferation and aggressive invasion into other organs, tumor

cells possess an extensive capacity for protein synthesis, mobility, and modification. Consequently, they typically exhibit a more advanced proteasome pathway compared with normal cells (Qu et al., 2021). Tumor cells rely on the proteasome system, which uniformly breaks down and reassembles functional proteins, to offset stress caused by the marked creation and alteration of proteins. Among the various types of tumor cells, MM cells have a greater reliance on the UPS due to their abundance of released proteins (Deshaies, 2014). Within MM cells, there is a notable accumulation of unfolded proteins during the processes of synthesis, proper folding, glycosylation, and transportation of newly synthesized proteins. These unfolded proteins consist of monoclonal immunoglobulins and various cytokines crucial for maintaining stromal cell interactions. The highly sophisticated endoplasmic reticulum–associated protein degradation (ERAD) systems in MM cells play a pivotal role in eliminating misfolded proteins. These systems function to expel misfolded proteins from the endoplasmic reticulum (ER), tag them through polyubiquitination, and subsequently subject them to proteolytic degradation at the proteasome. Thus, proteasome inhibition would lead to an imbalance in the ERAD system, which would then cause the ER to become overstressed and cause MM cells to undergo apoptosis sequentially (Hiramatsu et al., 2015).

4. Chemoresistance nature of MM

Chemoresistance in MM refers to the situation in which cancer cells within the MM tumor exhibit resistance to chemotherapy medications, hence reducing the efficacy of the treatment. Chemoresistance can be seen in MM, a malignancy of plasma cells that is usually located in the bone marrow, for several reasons (Siveen et al., 2014). First, drug metabolism, DNA repair mechanisms, cell cycle regulation, and apoptosis are all impacted by genetic and molecular changes in MM cells, which reduces the effectiveness of chemotherapy. Furthermore, interactions between the bone marrow microenvironment and immune cells, stromal cells, and extracellular matrix form a protective niche against chemotherapy, which plays a critical role in MM cell survival and resistance (Gourzones et al., 2019). To become more resistant to chemotherapy, MM cells can also acquire defense mechanisms such as drug efflux pumps, enhanced DNA repair, and modifications to apoptotic pathways. By controlling gene expression and promoting various susceptibilities to chemotherapy within the MM tumor, epigenetic modifications, clonal development, and heterogeneity further contribute to chemoresistance (Housman et al., 2014). In MM, the hypoxic bone marrow microenvironment offers several benefits for the initial phase of chemoresistance, which is depicted in Fig. 26.3. Because tumor cells rely more on proteasomes than normal cells do, they are more susceptible to proteasome suppression (Schwestermann et al., 2022). On the other hand, proteasome inhibitors (PIs) may become less effective as chemoresistance develops. Chemoresistance to PIs is readily induced by hypoxia stimulation, and there is a notable increase in the posttranslational expression of the steroid receptor coactivator 3 (SRC-3) (Guo et al., 2022).

This picture depicts the various factors that are associated with the chemoresistance nature of MM cells such as increased drug efflux, increased rate of DNA repair, controlled gene expression, modified apoptotic pathways, epigenetic modifications, increased PI resistance by altered drug targets, which have been induced by specific cell signals and stimuli released from hypoxic bone marrow.

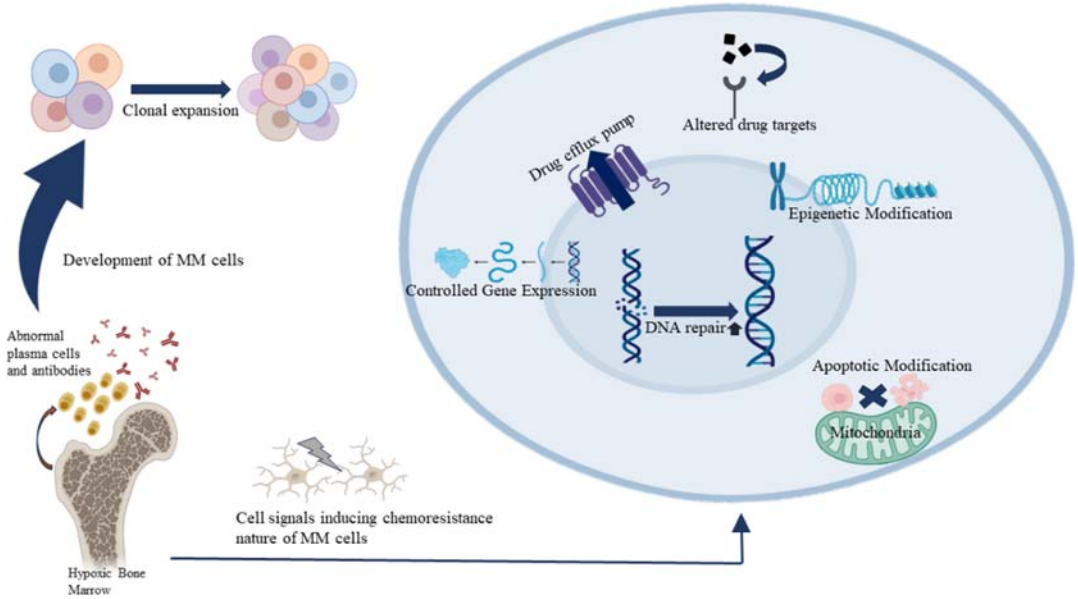

FIGURE 26.3 Chemoresistance nature of MM cells.

4.1 Pathophysiology involved in the chemoresistance nature of MM cells

The chemoresistance of MM cells is largely dependent on the proteasome, an essential cellular component. One of the mainstays of MM treatment is the inhibition of the proteasome by certain medications. By obstructing this cellular structure, proteasome inhibitors stop the degradation of proteins in MM cells. MM cells are more dependent on the proteasome pathway to break down particular proteins that are necessary for their survival and growth. Proteasome inhibition is an important treatment approach because it induces cell death in MM by interfering with these vital activities (Gandolfi et al., 2017).

MM chemoresistance is strongly linked to the UPS pathway, which is the most important mechanism for protein breakdown within the cells. In MM cells, this process is frequently dysregulated, leading to an overactive proteasome system that excessively breaks down important proteins. Proapoptotic proteins, cell cycle regulators, and tumor-suppressor proteins are examples of this. The ubiquitin-proteasome pathway's hyperactivity has a major role in the establishment and upkeep of chemoresistance in MM cells (Hideshima and Anderson, 2012).

In addition, the proteasome controls the NF-κB signaling pathway, which is essential for inflammation, cell division, and survival. Aberrant NF-κB activation is often seen in MM, where it enhances cell survival and resistance to treatment. By stopping the degradation of inhibitory proteins, inhibiting NF-κB activity, and increasing the susceptibility of MM cells to drugs that induce death, proteasome inhibition upsets this system (Ma et al., 2003).

Cyclins and CDKIs, two necessary cell cycle regulators, are also degraded in MM cells under the direction of the proteasome pathway. These cell cycle regulators build up and impair

the course of the cell cycle, ultimately leading to cell cycle arrest, by blocking the proteasome. As a result, this prevents the growth and multiplication of MM cells. By regulating the amounts of critical regulatory proteins such as cyclins and caspases in both normal and tumor cells, the proteasome is essential for intracellular protein degradation and plays a significant part in mediating several events related to cell survival and development (Santo et al., 2015). Fig. 26.4 depicts the factors that stimulate and inhibit the action of MM cells.

The left side depicts the growth and proliferation of MM cells, which has been induced by UPS and NF-κB signaling pathway along with the production of cyclins, IgG, etc., leading to the chemoresistance nature of MM cells, whereas the right side shows the factors that lead to the apoptosis of MM cells. Proteasome inhibitors and overstressed ERAD system lead to increased production of misfolded proteins, thereby increasing the unfolded protein response eventually causing the MM cells apoptosis.

The hallmark of MM is the overproduction of IgG in plasma cells, which exhibit increased levels of proteasome activity and are, therefore, more susceptible to proteasome inhibition than normal cells. As a deubiquitinase, PSMD14 exerts a positive influence on the initiation of the BMP6 signaling pathway by removing ubiquitin chains linked at K48 on ALK2. This action enhances tumor growth and chemoresistance by bolstering the stability of the ALK2 receptor (Jayaweera et al., 2021). Therefore, proteasomes are essential to the MM's development of chemoresistance. One important regulator of chemoresistance to PIs is hypoxia-induced SENP1, and other proapoptotic actions in cells are mediated by proteasome suppression. The complicated pathophysiology of proteasomes implicated in MM chemoresistance includes the regulation of cell survival and progression events, as well as the degradation of intracellular proteins (Seo et al., 2019).

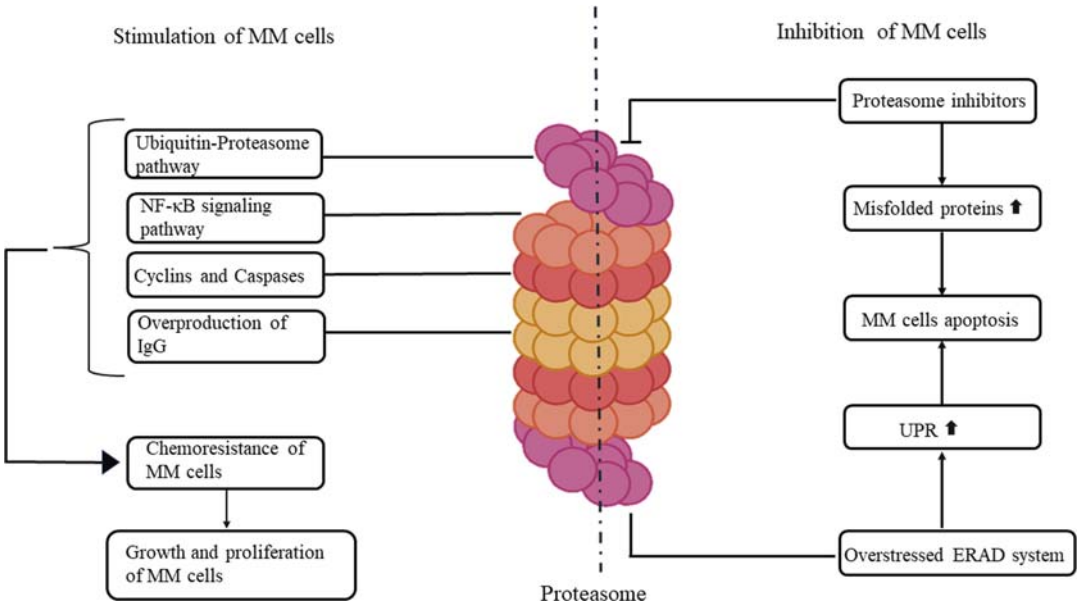

FIGURE 26.4 Stimulation and inhibition of MM cells.

5. The action of proteasome inhibitors on MM

Drugs that are referred to as proteasome inhibitors particularly target and block the cellular complex known as the proteasome, which breaks down proteins. These medications cause an accumulation of undesirable or hazardous proteins within the MM cells by blocking the proteasome, which stops proteins from being broken down. Since proteasome inhibition leads to a buildup of misfolded proteins, it triggers an unfolded protein response (UPR), which is essential to the cytotoxic action of PI. Therefore, the degree of MM cell's PI sensitivity may depend on how dependent they are on the proteasome and the UPR, which has been depicted in Fig. 26.4. PIs are now a recognized therapeutic option for the treatment of MM (Nunes and Annunziata, 2017). The effective introduction of bortezomib, a proteasome inhibitor, marked effective utility in cancer indications (Sriramulu et al., 2021). The FDA has approved bortezomib, the most well-known PI, for the MM treatment. By attaching to the 20S proteasome subunit, bortezomib inhibits the proteasome, causing ubiquitinated proteins to accumulate and eventually causing MM cells to undergo apoptosis (Hungria et al., 2019). Carfilzomib, ixazomib, and oprozomib are further PIs that have been developed for the treatment of MM. Preclinical and clinical trials have yielded encouraging results for these inhibitors, and some have even been given the go-ahead for clinical usage (Merin and Kelly, 2014).

5.1 Combinational therapies used in the treatment of MM

The discovery of drug compounds has a long history in drug repurposing, notably by fortuitous findings. It has taken a new path in the creation of novel therapeutics based on existent or authorized drugs in recent years (Shruthi et al., 2023). One important component of the treatment for MM has been proteasome inhibition. To increase effectiveness and decrease side effects, numerous combinations with different medicines have been investigated since the first PI, bortezomib, was approved (Ito, 2020). Proteasome inhibition-based combination treatments for MM include the following recent trends: lenalidomide, dexamethasone, and carfilzomib (KRd regimen): This regimen, when administered to newly diagnosed MM patients, has demonstrated a notable enhancement in both overall survival and progression-free survival when contrasted with the bortezomib, lenalidomide, and dexamethasone combination (VRd regimen) (Jasielec et al., 2020). The simple oral regimen of ixazomib, lenalidomide, and dexamethasone (IRd regimen) has shown positive results in relapsed or refractory MM patients (Minarik et al., 2021). Daratumumab in combination with lenalidomide, dexamethasone, and bortezomib (Dara-VRd) has shown improved depth of response and progression-free survival with this combination (Jakubowiak et al., 2022). HDACi panobinostat has been authorized for use in conjunction with dexamethasone and bortezomib in relapsed or refractory patients, and multimed therapy has undergone at least two previous regimens (San-Miguel et al., 2016). Marizomib is a novel proteasome inhibitor that is being studied and shows better results for treating multiple myeloma patients. In relapsed and refractory MM patients, it has been used with pomalidomide and low-dose dexamethasone (Naymagon and Abdul-Hay, 2016).

5.2 Biomarkers used in the identification of MM

Biomarkers are vital resources in the complex field of multiple myeloma, a cancer that originates from bone marrow plasma cells. These biomarkers have a variety of functions and offer a thorough framework for the management, characterization, and identification of multiple myeloma cells. Monoclonal immunoglobulins, or M protein, are a hallmark of multiple myeloma that are most commonly detected in a patient's blood or urine. In addition to confirming the existence of MM, this biomarker measures aberrant plasma cells, providing important information on the severity and course of the illness. Serum-free light chains (FLCs), namely kappa and lambda, and their ratio are measured in conjunction with M protein to further improve diagnostic precision. This is especially useful in situations, where the results of conventional M protein tests are unclear. From the original tumor location, circulating tumor cells (CTCs) are discharged into the peripheral circulation. Malignant PCs that had moved from the bone marrow to the bloodstream were identified as CTCs in MM. CTCs are therefore a promising biomarker for the detection of MM. Neoantigens are a class of antigenic products of tumor-specific somatic mutations that may one day be used as immunological biomarkers in MM. Because tumor-specific neoantigens do not cause autoimmune toxicity, neoantigen-specific T cell responses are consistently documented, which leads to their use as therapeutic targets for cancer immunotherapies (Soliman et al., 2021). Elevated blood levels of beta-2 microglobulin (β2M) are indicative of a tumor's burden and provide crucial prognostic information that informs therapy choices. An effective method for assessing cell surface markers such as CD38, CD138, and CD56 is flow cytometry, which provides information on malignant plasma cells (Solimando et al., 2019). Elevated levels of VEGF, IL-10, and IL-6 provide information about the interactions between MM cells and BM microenvironment. Bone lesions can be seen, and bone involvement can be evaluated using advanced imaging modalities such as positron emission tomography (PET) scans and magnetic resonance imaging (MRI). Testing for minimal residual disease (MRD) finds traces of remaining malignant cells, which is essential for assessing the effectiveness of treatment. This testing frequently uses flow cytometry, polymerase chain reaction (PCR), or next-generation sequencing. Monitoring serum creatinine and calcium levels is essential for treating problems associated with MM, including hypercalcemia and renal impairment. Fig. 26.5 depicts the flowchart of the various biomarkers used for the identification and characterization of multiple myeloma cells and also shows other treatment methods involved in the diagnosis of multiple myeloma cells. Overall, these several biomarkers improve the accuracy and efficiency of MM diagnosis (Giannakoulas et al., 2021).

5.3 Recent advancements undertaken for the treatment of MM

Several recent developments have considerably changed the landscape in the quickly developing field of treating MM, and various statement approaches have been discussed in Table 26.1. One of the hopeful applications of immunotherapies involves the

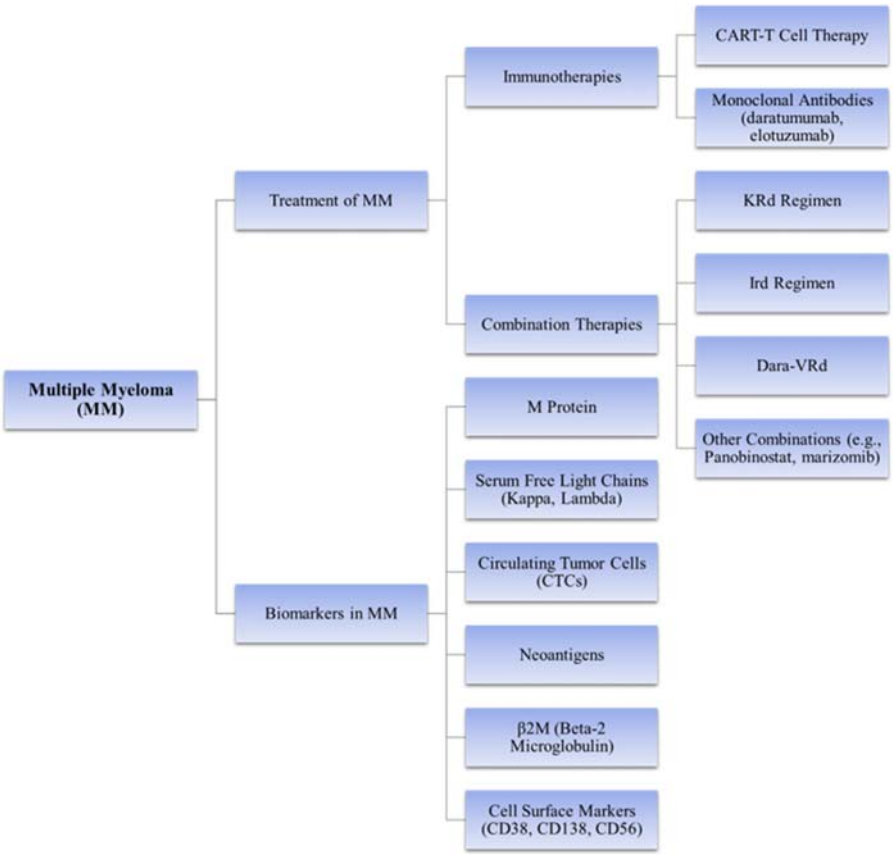

FIGURE 26.5 Treatment methods involved in multiple myeloma. This flowchart depicts various treatment methods involved in multiple myeloma such as immunotherapies and combination therapies and the biomarkers used in the identification and characterization of multiple myeloma.

modification of a patient's T cells to specifically identify and eliminate myeloma cells, with a particular focus on CAR-T cell therapy designed to target B cell maturation antigens (BCMA) (Demel et al., 2021). PIs and immunomodulatory drugs (IMiDs) are examples of targeted medicines that have shown efficacy through the disruption of vital cellular functions. Moreover, monoclonal antibodies that target particular proteins in myeloma cells, such as daratumumab and elotuzumab, have demonstrated promising results. PIs, IMiDs, MoA, and corticosteroids are frequently used in combination therapy, which is being used more for their combined therapeutic benefits (Davis and Sherbenou, 2021). Furthermore, developments in high-dose chemotherapy in conjunction with stem cell transplantation and techniques for assessing MRD are reshaping the medical field (Rein et al., 2013).

TABLE 26.1 Various treatment methods used in the diagnosis and the management of MM cells.

Treatment approach	Mechanism	Notable combination	References
Proteasome inhibitors	Target and block the proteasome, leading to protein accumulation in MM cells and induction of apoptosis.	Bortezomib, carfilzomib, ixazomib, oprozomib	Hungria et al. (2019); Merin and Kelly (2014)
Combinational therapies	Combining various drugs with proteasome inhibitors to enhance effectiveness and reduce side effects	Lenalidomide, dexamethasone, and carfilzomib (KRd)	Jasielec et al. (2020)
		Ixazomib, lenalidomide, and dexamethasone (IRd)	Minarik et al. (2021)
		Daratumumab in combination with lenalidomide, dexamethasone, and bortezomib (Dara-VRd)	Jakubowiak et al. (2022)
		HDACi panobinostat with dexamethasone and bortezomib	San-Miguel et al. (2016)
		Marizomib in combination with pomalidomide and low-dose dexamethasone	Naymagon and Abdul-Hay (2016)
Biomarkers for MM identification	Specifically used as an essential tool for the characterization and identification	M protein, serum FLC, CT, neoantigens, VEGF, IL-10, IL-6, serum creatinine	Giannakoulas et al. (2021); Soliman et al. (2021)
CAR-T cell therapy	Target B cell maturation antigens (BCMA)	Daratumumab, isatuximab, and elotuzumab	Demel et al. (2021)
Targeted medicines	Performs the disruption of vital cellular functions	Protease inhibitors and immunomodulatory drugs	Davis and Sherbenou (2021)
Monoclonal antibodies	Targets particular proteins in myeloma cells	Daratumumab and elotuzumab	Davis and Sherbenou (2021)

CT, circulating tumor; *HDACi*, histone deacetylase inhibitors; *IL 6*, interleukin 6; *IL-10*, interleukin 10; *VEGF*, vascular endothelial growth factor.

6. Conclusion and future directions

This chapter delves into the intricate roles of proteasomes in maintaining cellular homeostasis and regulating essential biological processes within normal cells. The ubiquitin-proteasome system emerges as a pivotal mechanism, orchestrating protein recycling, degradation, and transcription factor regulation. Proteasomes play a crucial part in cell cycle control, antigen processing, immune response, and the NF-κB signaling pathway. Understanding these functions provides critical insights into cellular processes. Focusing on multiple myeloma (MM), this chapter emphasizes the significance of proteasome inhibitors in combating this challenging and incurable cancer. MM's unique characteristics, rooted in the malignant transformation of plasma cells and the resulting aberrant protein accumulation, underscore the

criticality of proteasome inhibition in MM treatment. This chapter sheds light on the chemoresistance nature of MM and the underlying pathophysiology, highlighting the impact of hypoxic bone marrow microenvironments. The action of proteasome inhibitors on MM cells is detailed, showcasing how these drugs disrupt proteasome function, leading to the accumulation of harmful proteins and triggering apoptosis in MM cells. Notably, bortezomib and other proteasome inhibitors exhibit promising results in clinical trials, suggesting a positive outlook for MM treatment. This chapter concludes by underlining the evolving landscape of clinical trials and prospects, indicating the potential of combination therapies and novel proteasome inhibitors in improving outcomes for MM patients.

Promising avenues for upgrading patient outcomes in multiple myeloma treatment are ahead. Immunotherapies are gaining popularity and have the potential to become more broadly used and effective. One example is CAR-T cell treatment. More individualized treatment regimens may result from personalized medicine, which is fueled by biomarkers and genetic screening. Precision medicine may be made possible by developments in proteomics and genomes, and the use of combination treatments and novel drug classes may lessen the need for conventional chemotherapy. It is anticipated that techniques for early detection and monitoring will advance, as will the evaluation of minimal residual disease (MRD) to inform treatment choices. Further investigation into the biology and genetics of multiple myeloma may provide new targets for treatment.

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CHAPTER

27

Role of cysteine cathepsin and matrix metalloproteinases in progression and chemoresistance in ovarian and breast cancer

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1. Introduction

Proteolysis is one of the most essential biological processes that regulates many fundamental aspects of cellular functioning including protein trafficking, protein activation, protein degradation, catalytic modification, etc. These proteolytic reactions occur due to a specific set of enzymes known as proteases (Sevenich and Joyce, 2014). Proteolytic enzymes or proteases are crucial for all life forms. In human genome, proteases are the best defined and one of the largest families of enzymes. They are essential for protein synthesis and control composition of proteins shape, size, turnover, and also the utmost destruction. Under favorable environmental condition, each protease is very specific and cleaves after a particular amino acid sequence. Proteases can be classified into six groups based on the nature of the main amino acids on the active site region of proteases and the mechanism of cleavage of peptide bonds, namely, cysteine protease, serine protease, aspartate protease, glutamic acid as well as matrix metalloproteases (López-Otín and Bond, 2008).

The development and progression of cancer is facilitated by the effect of its surrounding stroma. Cancer cells functionally modify their environment by secreting different chemokines, cytokines, and other components. This results in remodeling of the surrounding environment, which is known as tumor microenvironment (TME). To maintain the different biological functions, it is necessary to have a balance between the proteases and the antiproteases. Imbalance between these leads to many diseases including cancer. Proteases are

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associated with different processes of tumor development and progression by getting involved in various cascades and networks of proteolytic reaction (Mason and Joyce, 2011). Cysteine proteases are present in cytosol as well as in lysosome. Under some pathological conditions, these types of proteases are secreted in some cell types. Cysteine protease helps in the intracellular protein catabolism, extracellular protein degradation, function of macrophages, and many other processes. Cathepsin cysteine proteases can break down both the extra- and intracellular proteins (Eatemadi et al., 2017). The extracellular activity of the cysteine protease mediates the cancer progression and facilitates metastasis into the nearby tissues; that's why, this type of proteases can be used as an anticancer drug target. Matrix metalloproteinases (MMPs) belong to the family of zinc-dependent endopeptidases. As their name suggests, these proteins help in degradation of extracellular matrix and plays an important role in various steps of tumor growth and angiogenesis (Gialeli et al., 2011). Level of cysteine cathepsins and MMPs are increased in various type of cancer. They degrade the extracellular matrix and help in epithelial−mesenchymal transition (EMT) of cancer.

Chemoresistance refers to the ability of cancer cells to become resistant to the effect of chemotherapeutic drugs. Over the time, cancer cells develop different mechanism to resist the toxic effects of the chemotherapeutic drugs and reduce the efficacy of treatment. Drug resistance is classified as intrinsic and acquired resistance. Intrinsic resistance also known as innate resistance occurs before the administration of drugs and happens due to inherited genetic mutations in cancer cell. However, acquired resistance occurs when the effectiveness of the anticancer drugs gradually reduces after treatment, and it is caused by the induction of secondary proto-oncogenes. There are several chemotherapeutic drugs available that target the particular proteases and help in diminishing cancer progression. But cancer cells evolve various mechanisms to resist the effects of those drugs and become resistant to the drugs. So, it is an urgent necessity to develop drugs that can target particular molecule of specific pathway and can decrease the promotion of cancer. Here, we have attempted to discuss the role of cysteine cathepsins and MMPs in breast and ovarian cancer progression and the mechanism of chemoresistance of the targeted drugs.

2. Role of cysteine cathepsin and MMPs in breast and ovarian cancer progression

2.1 Cysteine

2.1.1 Cysteine cathepsins in breast cancer progression

Cysteine cathepsins are a group of lysosomal peptidases and consist of 11 members, including cathepsin B, F, C, K, L, X, W, O, S, V, and H. Cysteine cathepsins plays important roles in protein and lipid metabolism, antigen presentation, and autophagy. In a broad range, these proteases are crucial for maintenance of normal tissue homeostasis. In case of breast cancer, activity of cysteine cathepsins is much higher than the normal tissue (Lah et al., 1992). In tumor microenvironment, cathepsin B (CTSB) is mainly found in the tumor cells, macrophages, fibroblast cells, and also in endothelial cells. Cathepsin B plays an important role during the metastasis of breast cancer cells to the lungs and to the bone. Cathepsin C

acts during the early stages of tumor progression, and this enzyme stimulates the activation of neutrophil and promotes cancer cell proliferation into the lung. Cathepsin K (CTSK) helps in the progression of breast cancer through the activation of MMPs that ultimately degrade the ECM and increase the invasiveness and metastatic properties of breast cancer cells. Cathepsin L (CTSL) is mainly found in tumor-associated macrophages as well as cancer cell. Its expression is positively correlated with the progression of breast cancer. Cathepsin S helps the breast cancer to cross the blood−brain barrier and metastasizes into the brain. Cathepsin V (CTSV) degrades the GATA-binding protein 3 in estrogen receptor−positive breast cancer cells, and the degradation of GATA3 is related to cellular proliferation and thereby leads to breast cancer progression (Linders et al., 2023).

2.1.2 Cysteine cathepsins in ovarian cancer progression

In case of ovarian cancer also, cysteine cathepsins plays a crucial part. In ovarian cancer, cysteine cathepsins are involved in degradation and remodeling of the ECM components and allow cancer cell invasion and metastasis. Cathepsin B, C, and S play important function in the progression of ovarian cancer (Ahn et al., 2010). Cathepsin L is also found to be highly expressed in ovarian cancer, where it contributes to the proliferation and metastasis of ovarian cancer (ZHANG et al., 2015). As cysteine cathepsins are upregulated in different types of cancer, these have emerged as an important molecular target in cancer treatment. Function of cysteine cathepsins and their inhibitors in different steps of cancer progression are depicted in Fig. 27.1.

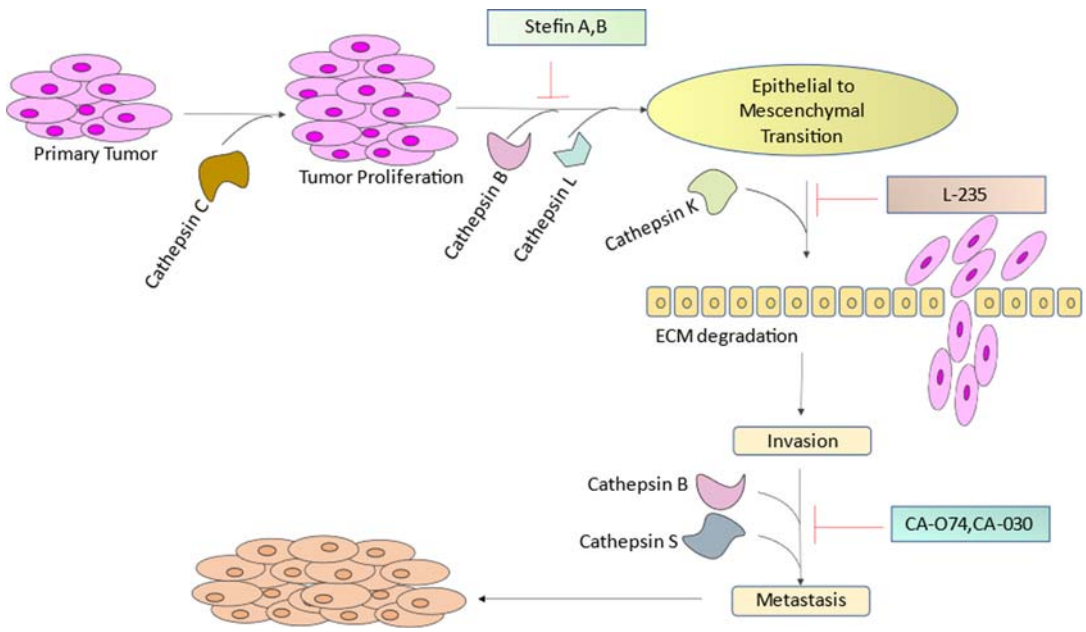

FIGURE 27.1 Role of cysteine cathepsins and their inhibitors used in different steps of cancer progression. *Modified from Tan et al. (2013).*

Cathepsin C promotes cancer cell proliferation, while B, L, K induce EMT, and this pathway can be inhibited by stefin A, B. Cathepsin K helps in ECM degradation, leading to invasion, and is inhibited by L-235, while cathepsin B and S promote metastasis, and this pathway can be inhibited by CA074 and CA030.

2.2 Role of MMPs in breast and ovarian cancer progression

Matrix metalloproteinases are one of the most important proteases associated with progression of various types of cancer. In TME, MMPs are produced by stromal cells as well as tumor cells and promote tumor progression by contributing tumor growth, invasion, and angiogenesis. There are about 23 MMPs identified in humans and are classified as collagenase (MMP 8, 13, 18), gelatinase (MMP 2, 9), stromelysins (MMP 3, 10, 11), matrilysins (MMP 7, 26), membrane type (MMP 14, 15, 16, 17, 24, 25), and other MMPs (MMP 19, 20, 21, 22, 23). Initially, it was thought that MMPs induce cancer by ECM remodeling, but there are several mechanisms discovered by which it can promote cancer (Cathcart et al., 2015).

In breast and ovarian cancer microenvironment, MMPs are expressed in different cell types such as immune cells, fibroblast as well as tumor cells. MMP 3, 9 induce EMT during tumor progression. Their protease activities help to degrade E-cadherin and other epithelial markers. MMP 3 induces EMT by upregulating the expression of SNAIL family transcription factors. MMP 2, MMP 9, and MMP 14 induced EMT by activating TGF beta pathway, which is a prominent signaling pathway in cancer (Kessenbrock et al., 2010). MMP 2, 7, 9, 14 are found in different stages of ovarian cancer (Brun et al., 2008). MMP 2 and MMP 9 were found to be secreted and activated during the progression of ovarian cancer and promote metastasis and invasion and related to poor survival. The upregulated expression of MMP 3 in ovarian cancer has been connected with the decreased expression of miRNA200. MiRNA200 mainly inhibits the invasiveness of ovarian cancer, but the overexpression of MMP 3 reduced the capacity of miRNA200 to suppress the invasiveness of ovarian cancer. Also, the expression of MMP 11 in ovarian cancer is positively correlated with the tumor stages (Al-Alem and Curry, 2015). Overexpression of MMP 14 induces the VEGF production and stimulates angiogenesis in breast cancer cell (Sounni et al., 2004). In breast cancer, MMP 1 promotes tumor growth and lung and brain metastasis in in vivo condition. MMP 2, 9, 14 enhance the migration and invasion of breast cancer. MMP 11 increases the breast cancer cell survival and promotes tumorigenesis in both in vitro and in vivo condition. MMP 7 helps to the proliferation of mammary gland and enhances tumorigenesis (Kwon, 2023). Roles of various MMPs in different steps of cancer progression are summarized in Table 27.1.

3. Cysteine cathepsins and MMPs in drug resistance

3.1 Overview of role of cysteine cathepsins in drug resistance

Over the past few decades, researchers have elucidated that there are a multitude of intricate mechanisms utilized by cancer cells to evade the cytotoxic effect of chemotherapy and

TABLE 27.1 Function of MMPs in different steps of cancer progression.

Steps of cancer progression	Matrix metalloproteinases	Inhibitors
Tumor proliferation	MMP 1, MMP 2, MMP 3, MMP 7, MMP 11, MMP 19	Rebimastat
Epithelial to mesenchymal transition	MMP 2, MMP 3, MMP 7, MMP 9, MMP 14	Batimastat and marimastat
Angiogenesis	MMP 1, MMP 2, MMP 3, MMP 7, MMP 9, MMP 13	Tanomastat

targeted therapy. Thus, understanding the mechanisms of drug resistance is important for developing new effective treatment strategies. In recent years, lysosomal cysteine cathepsins contribute to the development of drug resistance in various types of cancers. Lysosome is a membrane-bound organelle that contains many hydrolytic enzymes including cysteine cathepsins. Many chemotherapeutic drugs sequestered in lysosome where the drugs can be stored and later enter into the cell through endocytosis. Cysteine cathepsin may inactivate different drugs and decrease their effectiveness against cancer cell. The drug-resistant cancer cells have the ability to alter the lysosomal permeability that results the retention of drugs within lysosome. Inside lysosome, the pH is acidic, which is important for proper functioning of the lysosomal enzyme including cysteine cathepsins. If there is any change on the pH, the enzymes decrease their ability to process chemotherapeutic agents and many other components (Halaby, 2019). Cysteine cathepsins−mediated autophagy is also associated with the drug resistance. It involves degradation of damaged cellular organelles with the help of lysosome. In drug-resistant cancer cell, elevated autophagy can improve cell survival through the degradation of impaired organelles and facilitate stress induced by drugs (Rudzińska et al., 2019). There are many drugs accumulated into the lumen of lysosome and consequently cause the exocytosis of lysosome (Zhitomirsky and Assaraf, 2017) and cannot interact with their specific targets. Doxorubicin, sunitinib, daunorubicin, and mitoxantrone are some drugs that follow this phenomenon, and after internalization, they accumulated into the lysosome. Lysosomal exocytosis also occurs when the cells become more hypoxic and acidic in the TME. Due to the hypoxic condition, TME becomes acidic, which induces the secretion of cathepsin and other proteases into the extracellular space and increases the invasiveness of tumor. In a breast cancer study, it was found that after treatment with paclitaxel, macrophage infiltration in TME increased. This cancer-associated macrophage expressed cathepsin B and provided protection against taxol-induced death of tumor cell. Similar occurrence was reported after treatment with doxorubicin and etoposides (Shree et al., 2011). It has been indicated that gemcitabine and 5-fluorouracil stimulates the lysosomal permeabilization of myeloid-derived suppressor cells (MDSCs), which results in the release of cathepsin B. Then cathepsin B activates inflammasome, and MDSC secretes IL-1β and controls the anticancer properties of those drugs (Bruchard et al., 2013). In case of ovarian cancer, the expression of cathepsin L was increased in paclitaxel-resistant cells, and knockdown of cathepsin L increases the susceptibility of ovarian cancer cells to paclitaxel (Sui et al., 2016). In different tumor cell line, cathepsin L inhibits the process of cellular senescence and suppresses the identification of

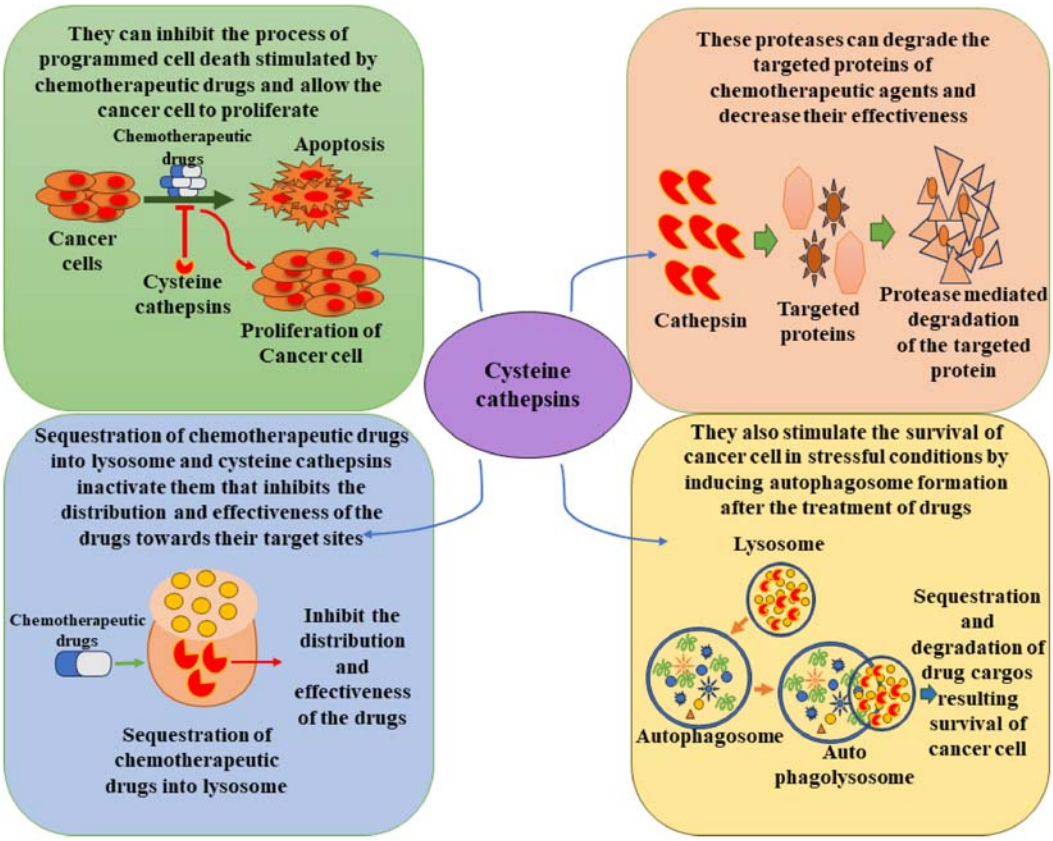

FIGURE 27.2 The mechanisms of chemoresistance driven by cysteine cathepsins. Different processes of drug resistance involving cysteine cathepsins.

protease-mediated drug target and ultimately traps the drugs into the lumen of lysosome. Inhibition of cathepsin L changes the resistance to several drugs (doxorubicin, tamoxifen, imatinib) by accumulating their specific protein targets (Topo-IIα, Erα, and Bcr-Abl) (Zheng et al., 2009). Even though the importance of cysteine cathepsins in drug resistance becomes more evident, still there is lack of in-depth understanding of the role and mechanism and rigorous and systematic study is needed for successful targeting of this chemoresistance mechanisms in human malignancies. The mechanisms of chemoresistance driven by cysteine cathepsins are presented in Fig. 27.2.

Cysteine cathepsins inhibit cancer cell apoptosis and enhance proliferation. The sequestration of chemotherapeutic drugs in lysosomes leads to their inactivation by cysteine cathepsins and disrupt distribution of drugs to the target cite. Cysteine cathepsins can degrade protein target of chemotherapeutic drugs and also stimulate cancer cell survival from drug-induced stress by inducing autophagosome formation.

3.2 MMPs in drug resistance

Another important player in the complex network of chemoresistance mechanism is matrix metalloproteinases. As MMPs are crucial component during cancer progression, it is necessary to understand the mechanisms through which MMPs are involved in chemoresistance, and it is essential to develop the novel therapeutic strategy to target this particular mechanism. EMT of cancer cells makes benign cells highly resilient against the chemotherapeutic drugs, and this contributes to the development of chemoresistance. MMPs are the important players in the EMT. During EMT of cancer cells, epithelial cells lose their integrity by reducing the expression of E-cadherin and increase expression of mesenchymal markers such as vimentin, fibronectin, and N-cadherin. SNAIL is the transcription factor that induces the process of EMT by enhancing the expression of MMP 2 and MMP 9. Decreased expression of E-cadherin is because of the activities of MMPs, which cleaves E-cadherin and forms soluble E-cadherin to promote EMT through the EGFR signaling pathway (David and Rajasekaran, 2012). MMP 9 promotes cancer cell invasion by inducing the TGF-β (Dong et al., 2019). There are several other mechanisms of chemoresistance associated with several MMPs. Due to the promoter hypermethylation of MMP 1, its expression is increased in MCF7 breast cancer cell, resulting in development of tamoxifen resistance (Kim et al., 2022). A study reported that after interaction with cancer-associated fibroblast, breast cancer cells become resistant to taxotere. Here, MMP 1 is found to be upregulated in cancer-associated fibroblast (Cui et al., 2018). Researchers have observed that MMP 1 causes 5-fluorouracil resistance in nasopharyngeal cancer cell by enhancing dihydropyrimidine dehydrogenase and thymidylate synthase (Song et al., 2019). MMP 1 along with MMP 2 and MMP 9 mediates chemoresistance of breast cancer cells after the treatment with adriamycin (Shen et al., 2017). MMP 2, which plays a pivotal role during metastasis of cancer, has also observed to be involved in chemoresistance. During cancer progression, different EMT markers SNAIL, vimentin, and MMP 2 increased that results in inhibition of apoptotic pathway and induces cell proliferation, and it causes EMT-induced chemoresistance. In 2021, a study reported that MMP 2 and MMP 9 were upregulated in breast cancer cell resistant to epirubicin, by inducing orosomucoid, a plasma glycoprotein known to be associated with inflammation (Qiong and Yin, 2021). MMP 2 is also found to be highly expressed in HeLa and ME180 cervical cancer cells resistant to paclitaxel (Liu et al., 2019). MMP 2 is also found to be upregulated in temozolomide-resistant glioblastoma cells. Nuclear factor erythroid 2−like-2 transcription factor (NFE2L2) binds with the promoter of MMP 2 and makes the cancer cell resistant to temozolomide. Combined treatment of temozolomide and diosgenin (inhibitor of NFE2L2) sensitizes the cancer cells toward therapy and also decreases the expression of MMP 2 (Rajesh et al., 2019). MMP 9 and MMP 14 both are major MMPs that promote invasion of cancer cells. There are many studies about the role of MMP 9 in cancer. MMP 14 is found in the gemcitabine-resistant pancreatic cancer cell, and this is achieved by the phosphorylation of ERK1/2. MMP 14 contributes to drug resistance by the inhibition of cell cycle arrest (Dangi-Garimella et al., 2011). MMP 14 also shows lesser response to chemotherapy in case of triple-negative breast cancer cells (Thakur et al., 2019). Upregulated MMP 9 is related to the increased amount of VEGF and decreased

expression of E-cadherin. This results in resistant to oxaliplatin (Gao et al., 2017). In colorectal cancer, MMP 9 induction results in 5-fluorouracil resistance by activating NF-κB pathway (Fukuyama et al., 2007). MMPs are also involved in breakdown of ECM and remodeling, which allows the cancer cells to become metastasized into other organs. This induces the cancer cells to escape from the effect of chemotherapeutic drugs. MMPs also induce the cell survival signaling pathways, and cancer cells become resistant to chemotherapy-induced cell death. However, MMP-mediated chemoresistance possesses a significant role in the management of different type of cancer. So, considering these mechanisms, MMP targeting becomes an important strategy to promote chemosensitivity. Mechanisms and contribution of MMPs in development of drug resistance in different cancer are summarized in Figs. 27.3 and 27.4.

MMPs contribute to cancer chemoresistance via enhancement of invasiveness and angiogenesis. MMPs can create physical barrier through degradation of ECM and activating alternative survival signaling pathways in cancer cells.

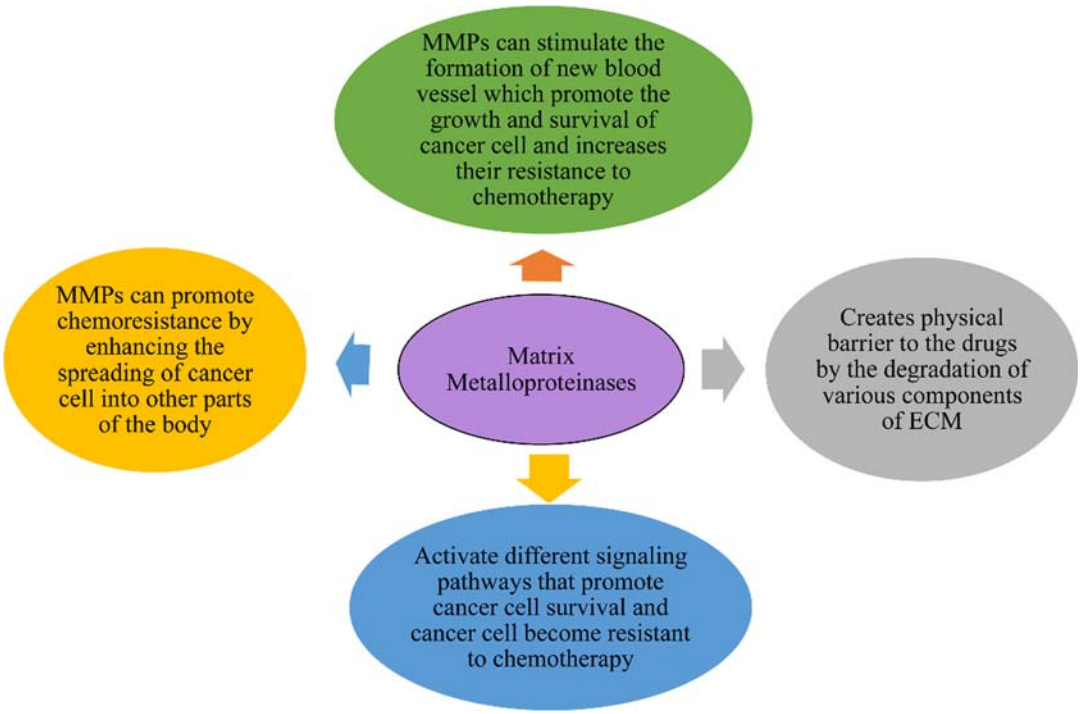

FIGURE 27.3 Matrix metalloproteinases—mediated chemoresistance in cancer. Drug resistance mechanism of matrix metalloproteinases.

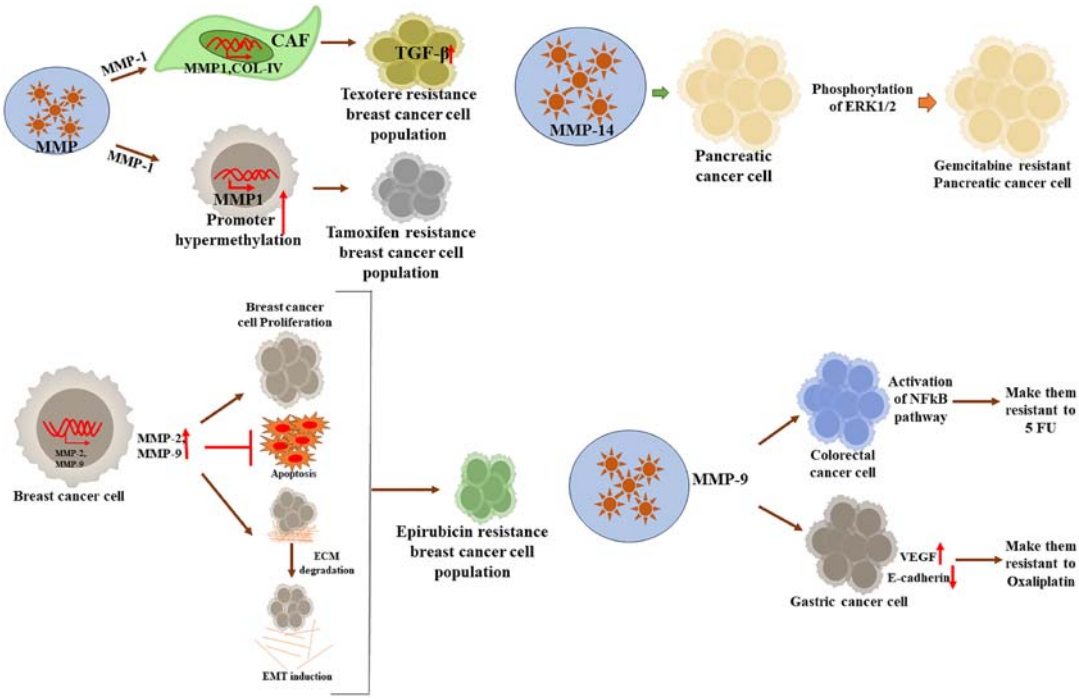

FIGURE 27.4 Role of different MMPs in regulation of drug resistance in cancer. Involvement of different MMPs in drug resistance in different cancer.

4. Targeting cysteine cathepsins and MMPs in regulation of chemoresistance and apoptosis in cancer

4.1 Overview of cysteine cathepsin–mediated regulation of programmed cell death and drug resistance in cancer

Cysteine cathepsins are highly expressed in several types of cancers. So, downregulating the activity of proteases can be considered as viable therapeutic strategy against cancer. Different therapeutic strategy has been developed such as antibodies, chemical inhibitors, gene therapy treatment that targets the activity of proteases and reduces the proliferation, angiogenesis, and invasion of cancer cells.

There are a variety of inhibitors that interact with the active site of cathepsins and reduce their activity, including cystatins, stefins, thyropins, serpins, and kininogens. Stefins and kininogens are the main cellular inhibitors of cathepsins, and cystatins are secreted from the cell. Stefin A and stefin B have the ability to reversibly block the activity of cathepsin B, H, L, S, and C. In breast and ovarian cancer, downregulation in the expression of stefin A and B are related with the development of cancer (Lah et al., 1992; Levicar et al., 2002; Kastelic et al., 1994). Several small molecules have been developed as lysosomal cysteine protease inhibitors, which targets the cysteine cathepsins activity such as CA-074 and CA-030 to inhibit

cathepsin B (Withana et al., 2012), and CLIK-148 and CLIK-195 to inhibit cathepsin L (Katunuma, 2011). E-64 inhibits the activity of cathepsins and reduces breast cancer and ovarian cancer metastasis (Leto et al., 1994). Nitroxoline also inhibits cathepsin B reversibly (Mirković et al., 2015). KGP-94 is one of the most important inhibitors of cathepsin L and decreases the invasiveness of breast tumor (Sudhan and Siemann, 2013). VBY-999 is a potent inhibitor of cathepsin S and inhibits the development of triple-negative breast cancer (Wilkinson et al., 2016). L-235, which is a potent inhibitor of cathepsin K, reduces breast cancer bone metastasis (Duong et al., 2014). Although these inhibitors show promising results, their efficacy and safety for the treatment of breast cancer are still under investigation. Cysteine cathepsins induce chemoresistance of cancer cells by enhancing drug efflux and activation of DNA repair mechanism. The inhibitors of cysteine cathepsins reduce the activity of cysteine cathepsins and decrease their involvement in drug reflux and DNA repair. After inhibition of this protease, cancer cells become more sensitive to chemotherapy, and these results enhanced accumulation of drugs inside the cells and improved therapeutic outcomes. In 2015, a study reported that suppressing the activity of cathepsins protects the neuroblastoma cells from doxorubicin-induced cell death and the cancer cells become sensitized toward doxorubicin (Gangoda et al., 2015). Researchers also found that paclitaxel-resistant A549 cells expressed TGFβ in a large amount. By suppressing TGFβ 3 in the paclitaxel-resistant A549 cells, expression of cathepsin L also reduced and the cells become sensitive to the drug. They also found that nuclear localization of Smad3 also increased in the paclitaxel-resistant A549 cells. Smad3 binds with the promoter region of cathepsin L, and inhibition of smad3 decreases the promoter activity of cathepsin L. So, cathepsin L regulates drug resistance through the activation of TGFβ/smad signaling pathway (Zhao et al., 2019) (Fig. 27.4).

MMP 1, collagenase-IV induces CAF that elevates TGF beta conferring taxotere resistance in breast cancer, and MMP promotor hypermethylation promotes tamoxifen resistance. MMP 14 can induce gemcitabine resistance in pancreatic cancer via activation of ERK1/2 pathway, and MMP 1 and 9 contribute toward epirubicin resistance in breast cancer by inhibition of apoptosis, ECM degradation, supporting proliferation and EMT, while MMP 9 can induce 5-FU resistance in colorectal cancer cell via activation of NF-κB pathway and oxaliplatin resistance in gastric cancer cells by upregulation of VEGF and downregulation of E-cadherin.

Cysteine cathepsins are also involved in the regulation of apoptotic cell death. These proteases cleave the proapoptotic factor Bid and Bax and also activate them. These proapoptotic proteins induce mitochondrial membrane permeabilization and stimulate the release of cytochrome C from mitochondria that ultimately results in caspase-dependent apoptotic cascade. Cysteine cathepsins also activate caspases, which are important players of apoptosis (Droga-Mazovec et al., 2008). TNFα and TNFR1 interaction also activates caspase 8, which induces lysosome to release the cathepsin B. This cathepsin B stimulates the release of cytochrome C that activates caspase 9 and caspase 3 and ultimately results in apoptotic cell death (Guicciardi et al., 2000). Although many chemotherapeutic drugs are used to induce apoptosis, continuous administration of those drugs enhances the risk of drug resistance. How cysteine cathepsins regulate drug resistance and apoptotic cell death is summarized in Fig. 27.5.

CTSL promotes paclitaxel resistance in lung cancer via upregulation of TGF beta and ECM degradation, leading to EMT induction; SMAd3 activation by CTSL induces proliferation, migration, and survival of paclitaxel-resistant lung cancer cells. Contrastingly, TNFr1/TNF alpha signaling can induce caspase 8−mediated release of cathepsin B that

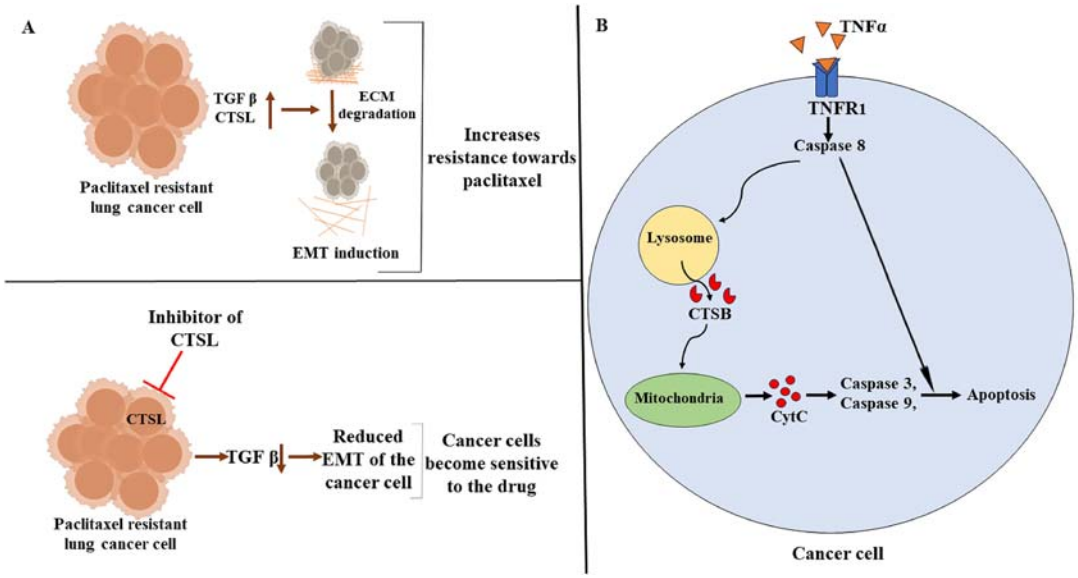

FIGURE 27.5 Mechanism of regulation of cancer cell drug resistance and apoptotic cell death by cysteine cathepsins. Cysteine cathepsin in regulation of chemoresistance and apoptosis.

can promote apoptosis in cancer cell via cytochrome *c* release from mitochondria that activates caspase 9 and 3.

4.2 Targeting MMPs in regulation of chemoresistance and apoptosis in cancer

MMPs are one of the important factors that are increased during tumorigenesis, and therefore, they represent important target for cancer therapy. Due to involvement of MMPs in EMT during cancer progression, which leads to chemoresistance, suppressing the activation of MMPs may be a promising approach for the treatment of cancer to the cytotoxic drugs. This process increases the sensitivity of cancer cells toward the effects of the chemotherapeutic drugs. Several MMP inhibitors have been described that inhibit various types of cancers. The broad-spectrum MMP inhibitors such as marimastat (BB-2516) and batimastat (BB-94) have been investigated for clinical trials in breast cancer. They target MMP 2 and MMP 9 and reduce cancer metastasis (Rasmussen and McCann, 1997). Batimastat and marimastat both are hydroximate type of inhibitors and target MMP 1, 2, 3, 7, 9 and reduce breast cancer as well as ovarian cancer. Initial trials results of the drug batimastat showed decreased oral bioavailability due to the lack of solubility in water. But direct injection of this drug into the peritoneal space shows efficacy with few side effects. Thus, another orally bioavailable drug marimastat was developed, which acts through similar mechanism of batimastat. It shows promising results in several cancer treatments, but there are also issues of low survival rate. Tanomastat, rebimastat, and prinomastat are also small molecule selective inhibitors of MMPs. Tanomastat inhibits the function of MMP 2, 3, 8, 9, 13; prinomastat inhibits the function of MMP 2, 3, 9, 13, 14;

and rebimastat inhibits MMP 1, 2, 3, 8, 9, 13, 14. These inhibitors reduce the metastatic progression of breast, ovarian, and many other cancers (Winer et al., 2018). The proteolytic activity of MMPs is regulated by some endogenous protein inhibitors known as tissue inhibitors of metalloproteinases (TIMP). TIMP family consists of 4 members such as TIMP 1, 2, 3, 4. They play a pivotal role in shaping the impact of ECM, cell adhesion molecules, various cytokines, chemokines as well as growth factors on cellular phenotype. Activity of MMPs can also be inhibited by some nonspecific inhibitors such as thrombospondin 1, 2, and α2-macroglobulin. Evaluation of TIMPs and thrombospondins and specifically designed drug delivery system in preclinical and clinical trial setup might provide us with novel antimetastatic therapeutic intervention strategy with reduced drug resistance in case of malignant neoplasia. There also some nonanticancer drugs that sensitize the cancer cell in combination with some anticancer drugs. Metformin is used to control type 2 diabetes and also inhibit the expression of MMP 9. In hepatocellular carcinoma, combinatorial treatment of metformin and sorafenib enhances the chemosensitivity of hepatocellular carcinoma cells to sorafenib treatment. Metformin suppresses the activity of MMP 9 through the downregulation of ERK1/2 and JNK1/2 (Hsieh et al., 2014). So, the downregulation of MMPs enhances the responsiveness of cancer cells to the anticancer drugs. In case of colorectal cancer, cotreatment of enalapril and 5-fluorouracil downregulates the expression of MMP 2 and MMP 9 by inhibiting NF-κB/STAT3 pathway and here enalapril increases the susceptibility of cancer cells to 5-FU chemotherapy (Yang et al., 2020). In 2020, Kosowska et al. found that sitagliptin (used to treat diabetes) enhances the responsiveness of ovarian cancer cells to chemotherapeutic drug paclitaxel. Cotreatment of these two drugs reduces the expression of MMP 1, 2, 7, 10 in ovarian cancer cells. Sitagliptin also activates caspase 3/7−mediated apoptotic cell death in ovarian cancer cells treated with paclitaxel (Kosowska et al., 2020). Several miRNAs interact with MMPs and promote EMT, angiogenesis, and remodeling ECM during cancer progression. So, interaction between miRNA and MMPs may be associated with drug resistance during cancer progression. In a study of glioblastoma, miR-211 induces apoptotic cell death and reduced proliferation, invasion of cancer cell through the downregulation of MMP 9. Combination of miR-211 and shRNA targeting MMP 9, in addition to temozolomide, increases apoptotic DNA fragmentation of glioma cancer stem cells and overcomes chemoresistance (Asuthkar et al., 2012). MMPs also regulate apoptosis in cancer and enhance chemoresistance. MMP 2 and MMP 9 activate PI3K/AKT signaling pathway that promote cell survival and inhibit apoptosis of cancer cell (Gu et al., 2021). So, increased expression of MMPs in cancer cells makes them less responsive to chemotherapy. MMP 7 cleaves Fas ligand from the cell surface and activates antiapoptotic signals of cancer cells. This phenomenon leads to the inactivation of Fas receptor and results in the development of resistance to apoptosis and makes the cancer cells become resistant to the drugs. MMPs also induce the secretion of several growth factors and cytokines from ECM that activate antiapoptotic pathways, thereby enhancing the resistance of cancer cells to the apoptotic effects of chemotherapy (Gialeli et al., 2011). A brief compilation of various protease inhibitors and state of preclinical and clinical study is summarized in Table 27.2.

TABLE 27.2 Protease inhibitors and their status of preclinical and clinical study.

Serial No.	Protease inhibitors	Clinical status of the drug	Targeting proteases	References
1	Stefin A and stefin B	Clinical	Cysteine cathepsin B, H, L, S, C	Lah et al. (1992), Kastelic et al. (1994)
2	CA-074, CA-030	Experimental	Cathepsin B	Withana et al. (2012)
3	E−64	Experimental	Cathepsin L, cathepsin D	Leto et al. (1994)
4	Nitroxoline	Experimental	Cathepsin B	Mirković et al. (2015)
5	KGP-94	Preclinical	Cathepsin L	Sudhan and Siemann (2013)
6	VBY-999	Preclinical	Cathepsin S	Wilkinson et al. (2016)
7	CLIK-148, CLIK-195	Preclinical	Cathepsin L	Katunuma (2011)
8	L-235	Preclinical	Cathepsin K	Duong et al. (2014)
9	Marimastat and batimastat	Investigational Experimental	MMP 1, 2, 3, 7, 9	Rasmussen and McCann (1997)
10	Tanomastat	Investigational	MMP 2, 3, 8, 9, 13	Winer et al. (2018)
11	Rebimastat	Investigational	MMP 1, 2, 3, 8, 9, 13, 14	Winer et al. (2018)
12	Prinomastat	Investigational	MMP 2, 3, 9, 13, 14	Winer et al. (2018)

5. Conclusion

Resistance to chemotherapeutic drugs remains a challenge for cancer treatment that often results in failure of treatment and reduces patient survival rates. The drug resistance of cancer cells develops because of the decreased drug availability and various oncogenic signaling pathways. These are regulated by specific factors of the tumor environment. Proteases are an important enzyme that mediates chemoresistance and promotes several types of cancers. By targeting the proteases, the process of drug resistance can be reversed. There are several small molecule inhibitors of cysteine cathepsins, and MMPs have been developed and tested in clinical and preclinical studies. Due to the small size and low molecular mass of these small molecular inhibitors or peptides, there is a challenge of the metabolic stability of these inhibitors (Li and Kang, 2020). Several protease inhibitors have failed due to their reduced survival rate; only few inhibitors are approved for the treatment. Using the approved drugs in combination with other DNA-damaging drugs may be beneficial for the breast, ovarian, and other types of cancer treatment. But there are few limitations that include low oral bioavailability, low retention time inside the body, low membrane permeabilization, quick renal clearance, etc. (Rudzińska et al., 2021). To overcome these problems, the nanoparticle-based drug delivery system has been developed as the new strategy for the protease-targeted therapy in cancer. Nanoparticle-based drug delivery system by targeting specific proteases improves bioavailability, enhances circulation time, and acts on the

particular tissue. Although the nanoparticle-based drug delivery system improves the survival rate, there are also some challenges including complex designing of the nanocomplex, toxicity and biocompatibility of the nanocomplexes, enzyme-substrate compatibility, diversity and complexity of the tumor-associated proteases, etc. (Yao et al., 2020). Further investigations are needed, and this may help to increase the survival rate. In conclusion, designing of the novel protease inhibitors and novel delivery system is very much crucial for more effective, safer, and patient-specific treatment strategy against progressive malignant neoplasia.

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CHAPTER

28

Proteases, chemoresistance, and cancer

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1. Introduction

Cancer is a term used to describe an assembly of diseases that are connected to uncontrolled cell progression and the production of abnormal amounts of cells in the body. Cancer is a significant health issue and one of the major causes of death (Eatemadi et al., 2017). However, today, we can diagnose and treat cancer effectively. Depending on the type and stage of cancer, numerous treatment options are available today, including chemotherapy, immunotherapy, radiation therapy, surgery, and combinations of these modalities.

2. Proteases

Proteases belong to the group of enzymes that are involved in the breakdown of proteins in the body. These play a very essential role in cell growth, tissue remodeling, immune responses, and many physiological actions in the body. Proteases are a wide range of enzymes distribution. There are about 990 protease genes identified in humans, and about 1605 protease inhibitory genes have been stated in humans. However, any disturbance in proteases may cause deregulation in the body's normal functioning. However, abnormal activation of proteases in cells and tissue may lead to cancer in the

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respective organs of the body (Song et al., 2021). Following are some examples of proteases that could be involved in cancer development (Lecaille et al., 2002).

2.1 Cysteine protease

Cysteine proteases fit into a major class of enzymes that belong to a larger group of proteases. These are involved in the itemization of proteins by cleaving peptide linkage (Verma et al., 2016). Cysteine proteases have a very reactive cysteine amino acid in their active site, and this residue plays a crucial role in their catalytic mechanisms. It has a very reactive thiol group (hydrogen removed from histidine). They use all their S^- ions as an attacker (nucleophile) to hydrolyze the peptide linkage (Fig. 28.1) (Buttle and Mort, 2013).

The cysteine proteases family contains papain, cathepsins, caspases, and calpains. A well-known cysteine protease produced from papaya, papain, is not only practiced for a variety of industrial purposes but also used in healthcare industries (Roy and Walsh, 2014). Cathepsins are a group of lysosomal enzymes that are linked to intracellular protein degradation. Abnormal expression and overactivation of cathepsins, especially cathepsin-B, -D, and -L, are involved in cancer invasion, metastasis, and tumor progression. Caspases are cysteine proteases that participate in programmed cell death. Calpains are calcium-dependent proteases that are associated with the remodeling process of the cell (Yadati et al., 2020). Member enzymes of the cysteine proteases family are explained in Fig. 28.2.

The cysteine proteases: cathepsin B, cathepsin L, and cathepsin S are known to be associated with the degradation of proteins related to extracellular matrix. This activity permits cancerous cells to assault surrounding tissues. By this activity, these can form metastases and promote tumor growth (Rudzińska et al., 2019). Cysteine proteases can facilitate the establishment of new blood vessels in tumors, this procedure is called angiogenesis. The degradation of extracellular matrix components normally inhibits blood vessel growth. This process helps tumors to attain the necessary nutrients and oxygen for their growth and metastasis. Cysteine proteases can modify the immune response by degrading the

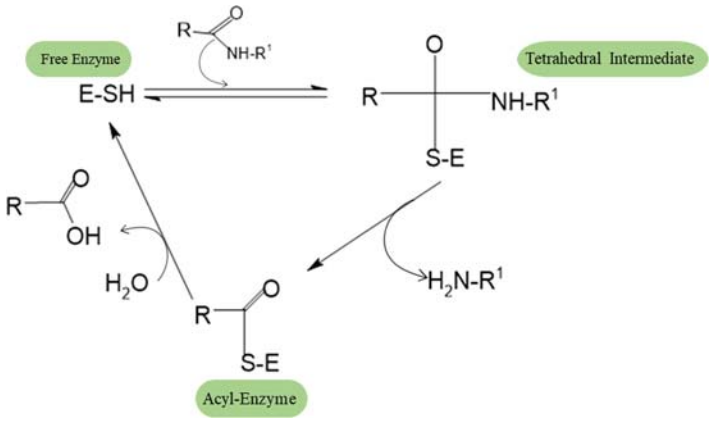

FIGURE 28.1 Cysteine protease action: Peptide bond cleavage and acyl-enzyme regeneration mechanism facilitated by histidine and cysteine at the active site.

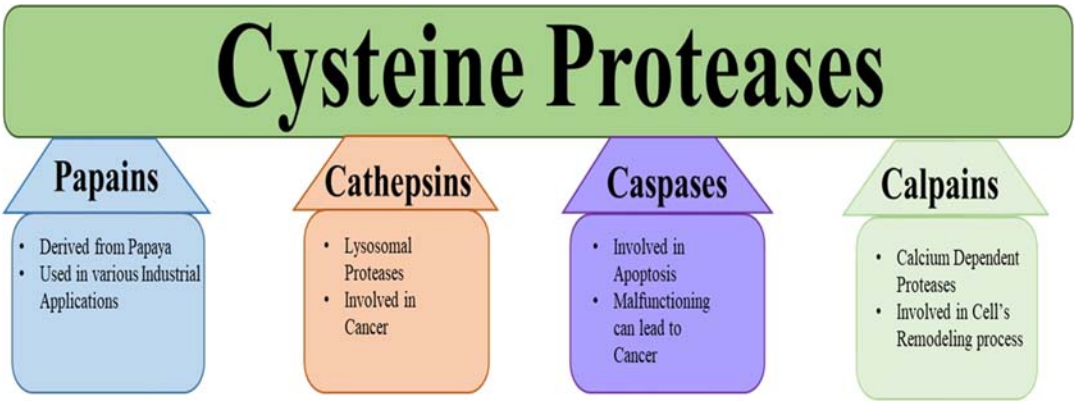

FIGURE 28.2 Schematic representation of cysteine proteases classification, featuring main enzymes such as papain, cathepsins, caspases, and calpains.

components of the immune system (Koblinski et al., 2000). As an example, they can degrade chemokines, cytokines, and major histocompatibility complex molecules. They can participate in impairing the ability of immune cells to distinguish and eradicate cancer cells. Cysteine proteases can prevent cancer cells from experiencing programmed cell death or apoptosis. They can cut and inactivate proapoptotic factors, which can inhibit the normal apoptotic signaling pathways and promote cell survival. Cysteine proteases released by cancerous cells can adjust the tumor microenvironment, tissue remodeling, angiogenesis, and promoting inflammation. This modified microenvironment can promote tumor growth, invasion, and metastasis (Vidak et al., 2019).

Cysteine proteases are targeted by pharmaceutical researchers because of their involvement in diseases. Inhibitors of cysteine proteases have been developed as therapeutic agents to modulate their activity and treat various conditions relating to their dysregulations (Verma et al., 2016). Iodoacetate is an irreversible inhibitor of papain (Satyanarayana, 2021). A lot of work has been done in this field, but still some more research is required to completely understand the complexity of cancer and its sure treatment.

2.2 Serine protease

Serine proteases belong to a set of enzymes that are related to the protease family and utilize a serine residue within their active site to catalyze the hydrolysis of peptide bonds. In several physiological processes, including digestion, blood coagulation, immunological responses, and tissue remodeling, they play critical roles. However, abnormal serine protease regulation has been linked to a number of illnesses, including cancer (Wu et al., 2021).

The mechanism of action of serine proteases includes a specific catalytic triad and contains three amino acids: serine, histidine, and aspartate. Serine proteases are initially synthesized in the form of inactive precursors known as zymogens (proenzymes). These zymogens or proenzymes are activated as a response to specific signals, such as proteolytic cleavage or binding to cofactors. Activation includes removal of an inhibitory segment from enzyme and revealing the active site. Once the enzyme is activated, the serine proteases can bind to their

specific substrate molecule. The activity site of these enzymes is a groove or pocket that accommodates the target substrate. Substrates usually have specific amino acid sequences that are acknowledged by the proteases. The catalytic triad assumes a character in the itemization of peptide bonds within the substrate molecule. This triad contains a serine residue (nucleophile), a histidine residue (base), and an aspartate residue (acid). These three residues are situated close to each other in the activity site of the enzyme. The hydroxyl group of serine residue (Ser) that is present at the catalytic triad acts as an attacker (nucleophile). It assaults on the peptide bond's carbonyl carbon within the substrate, and an intermediate (covalent acyl-enzyme) is produced. These clues to the breakdown of the peptide linkage and the N-terminal portion of substrate are released. The histidine residue (His) in the catalytic triad assists as a base. It accepts a proton from the serine hydroxyl group. This DE protonation boosts the nucleophilicity of the serine residue and permits it to bombard the peptide bond effectively. The aspartate residue (Asp) in the catalytic triad acts as an acid. It provides a proton to the departing amino group of C-terminal portion of the substratum. This proton transfer facilitates the cleavage of protein linkage, and the carbon side fragment is released. In this last step, after catalysis, the products are released (Fig. 28.3). Now the enzyme is able to bind and cleave some other substrate molecules (Radisky et al., 2006). The serine protease can experience multiple catalytic cycles, contributing to efficient and rapid processing of substrates (Voet and Voet, 2021; Bizzozero and Dutler, 1981). The overall mechanism of action of serine proteases comprises the nucleophilic attack of a serine residue, assisted by aspartate and histidine residues in the catalytic triad. This catalytic process assists the selective cleavage of peptide bonds within substrate molecules (Cen et al., 2019).

Its normal functioning is very beneficial for the normal physiological functioning of the body. But sometimes it is deregulated and can cause inappropriate functioning of the body. Due to this, it could be involved in progression of cancer (Tagirasa and Yoo, 2022). Serine proteases, such as uPA (urokinase-type plasminogen activator) and tPA (tissue type plasminogen activator), can activate plasmin, a serine protease that degrades constituents of the extracellular matrix (ECM) (Goretzki et al., 1992). Serine proteases may stimulate angiogenesis (production of new blood vessels) by activating matrix metalloproteinase (MMPs) (Kryza et al., 2018). MMPs degrade the ECM and release proangiogenic factors and enables the growth of new blood vessels that are used for the supply of oxygen and nutrients to the tumor (Falkowski et al., 2020). Serine proteases can modify the immune response in cancer. For example, granzymes (a family of serine proteases) released by cytotoxic T lymphocytes (CTLs) boost apoptosis in target cells, including cancer cells. Furthermore, serine proteases can cleave and inactivate immune molecules, impairing immune cell function and stimulate tumor immune evasion. Serine proteases can activate cell surface receptors called PARs, which are present on several cell types, including cancer cells. Activation of PARs by serine proteases triggers signaling pathways that support tumor growth, invasion, and angiogenesis. Serine proteases released by cancer cells can stimulate the tumor microenvironment. For instance, they can activate growth factors, cytokines, and chemokines, leading to inflammation, tissue remodeling, and as a result, it can enhance the tumor cell survival and proliferation (Shahinian et al., 2014).

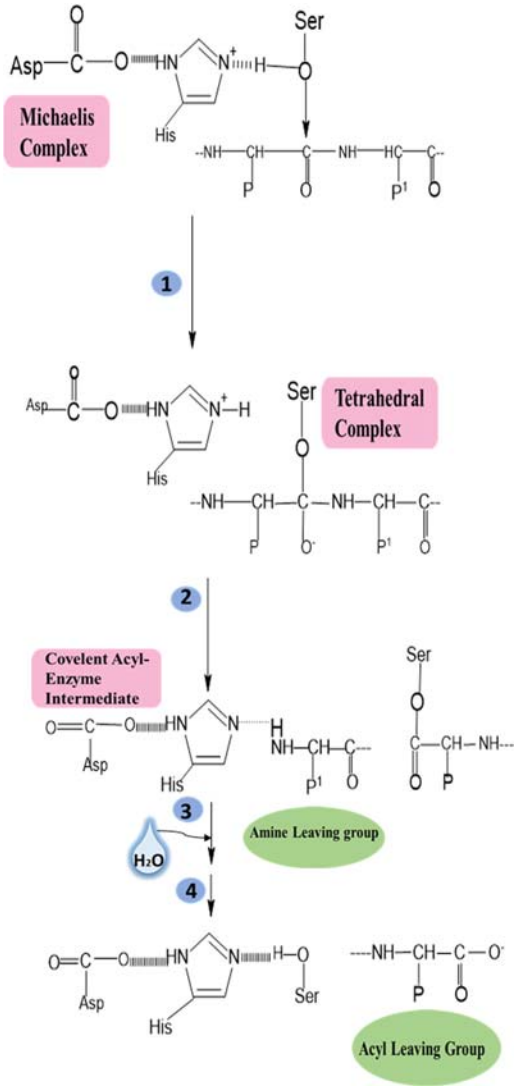

FIGURE 28.3 Mechanism of action of serine proteases that operate via a distinctive catalytic triad consisting of three crucial amino acids: serine, histidine, and aspartate.

2.3 Matrix metalloproteinases

MMPs are linked to cluster of enzymes and participate in degrading some protein constituents of the extracellular matrix (ECM), which allows cancer cells to attack surrounding tissues (Lecaille et al., 2002).

Overexpression of these enzymes (mostly MMP-2 and MMP-9) have been detected in several types of cancers. MMPs are a family of enzymes that play a vital role in the crushing and remodeling of the extracellular matrix, which are the noncellular components of tissues

(Mason and Joyce, 2011). These are the endopeptidases that are zinc-dependent and are participants of numerous physiological and uncontrolled processes, involving tissue development, wound healing, inflammation, and cancer progression (Liu and Khalil, 2017).

There are more than 20 known candidates for the MMP's family; every single member has different substrate specifications and cellular localization.

3. Chemoresistance

Chemoresistance, sometimes referred to as chemotherapy resistance, describes a cancer cell's capacity to fend off the effects of chemotherapeutic medications. Chemotherapy is a popular cancer treatment that uses medications to either kill or slow the development of cancer cells. However, some cancer cells can create defense against chemotherapy, which can result in treatment failure and disease progression. This is the capability of cancerous cells to resist against the drugs or any harming material to survive. Chemotherapy is a common method to fight against cancer. It can inhibit their growth, but some cells become resistant in some ways (Raguz and Yagüe, 2008). Chemoresistance occurs when responding cancer cells suddenly start grow and even survive under the effect of drugs. There are many factors that participate in chemoresistance in cancer cells (Fig. 28.4). These could transfer their gene of resistance to other cells. The resistant cells can secrete different proteins that could pump out the drugs or may inhibit the effective entry in the cell. These factors affect the cell in different ways at different levels. By using these factors, cells become resistant.

3.1 Overactivity of efflux pump

Overexpression of proteins is one of the major causes of chemoresistance. It actively pumps the chemotherapeutic drugs out of the cells and reducing the effectiveness and efficacy of drug. These proteins are ATP-binding cassette (ABC) transporters, which act as efflux pumps and involve in decreasing the concentration of drugs inside the cell. These involve decrease in the intracellular concentration of chemotherapeutic drug and limiting their cytotoxic effects. These are ATP-dependent protein pumps. These bind to ATP and

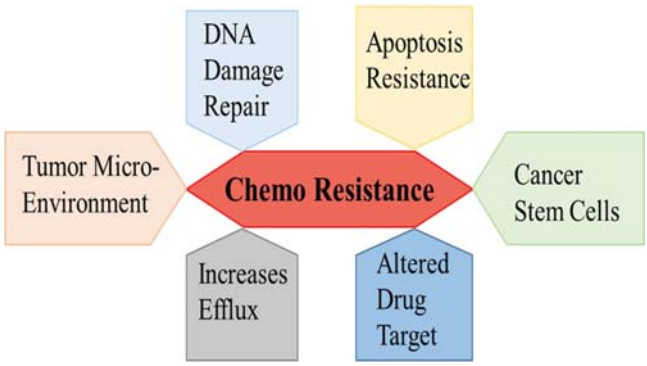

FIGURE 28.4 Schematic representation of significant factors that participate in chemoresistance in cancer cells.

involve in the activity of extracellular transport of the drug (Mohammad et al., 2018). These proteins (located in the cell's outer layer) bind to the drug regimen, use ATP, and expel the drug out of the cell. This is due to the mutation in the protein pump of the cell. Proteases are intricate in the regulation of activity of drug efflux pump. When proteases mutate, the concentration and effectiveness of drug is reduced in the cancerous cell. Misregulation of these proteases can cause modulation of these pumps; as a result, the cancer cells show reduced vulnerability to drug regimen-induced cytotoxicity (Wasaznik et al., 2009).

3.2 Inactivation of drug

Cancer cells can develop mechanisms to break down or inactivate chemotherapy drugs, reducing their effectiveness. They may produce higher levels of certain enzymes that can metabolize the drugs, or they may activate drug efflux pumps that expel the drugs from the cells before they can exert their effects (Gong et al., 2006). Proteases are majorly involved in the mechanism of drug modulation in the cell; thus if they are mutated, they can degrade the drug inside the cell, even before they reach the target. Some proteases such as metalloproteinases are involved in drug inactivation or degradation. They prevent them from reaching their target and exert their effect in cancerous cells (Jabłońska-Trypuć et al., 2016).

3.3 DNA repair mechanism

Chemotherapy drugs often work by promoting DNA destruction in cancerous cells, leading to cell death. However, resistant cancer cells can enhance their DNA repair mechanisms, effectively repairing the damage caused by chemotherapy and allowing them to survive and proliferate. Many proteases can regulate the DNA repair mechanism. These proteases prevent the cancerous cells from destruction. When drug induces DNA damage to cancer cells, Proteases play their role by assisting the repair mechanism and allowing the tumor to proliferate despite of the drug's presence and resulting DNA damage (Wei et al., 2021).

3.4 Apoptosis

Apoptosis is a planned (programmed) cell death process that is triggered by the stimulus from DNA damage or other cellular stress. Proteases, such as caspases, have a dominant role in regulating apoptosis (programmed cell death). Mutations in the process of apoptosis are an assurance of cancer. Proteases can modify the apoptotic pathways to encourage cell survival, even if the drugs are being there. Cancer cells can survive by angiogenesis or by making new blood vessels. These new blood vessels can increase the blood supply to the cells (Gordon et al., 2010). Chemoresistance can occur when cancer cells alter the targets that chemotherapy drugs act upon. For example, cancer cells can downregulate or mutate the proteins targeted by the drugs, making them less susceptible to their effects (Park et al., 2020).

3.5 Cell signaling pathways

Chemoresistant cancer cells may acquire mutations or alterations in the signaling pathways that regulate apoptosis, thereby evading cell death induced by chemotherapy. Proteases have the capability of activating many signaling pathways. These pathways can stimulate the cell survival by causing the resistance against chemotherapeutic drugs. For example, proteases can regulate (activate or block) the growth factors and their receptors (such as epidermal growth factors), causing an increase in cell survival and chemo resistance. Several studies show that the metalloproteinases are required for the activation of many G protein−coupled receptors and epidermal growth factors (Fischer et al., 2003).

3.6 Tumor microenvironment

The tumor microenvironment also participates in chemoresistance. Factors present in the tumor microenvironment, such as low oxygen levels (hypoxia), abnormal blood supply, pH adjustment, and interactions with other cell types, can create a protective niche for cancer cells, making them less susceptible to chemotherapy (Asgharzadeh et al., 2017). Proteases have the ability to modify the microenvironment around the cancerous cells. These produce a physical barrier around the cancerous cells that reduces the perception into the target cells. Some proteases (MMPs) are intricate in the degradation of extracellular matrix. These facilitate the production of thick, solid, and shielding stromal layer that causes the interference in drug delivery (Jabłońska-Trypuć et al., 2016).

Some researchers believe that chemoresistance can be attributed to a small subset of cells within tumors called cancer stem cells. These cells possess self-renewal and differentiation capabilities and are thought even more resistant to chemotherapy than other cancer cells. If chemotherapeutic agents selectively target the bulk of the cancerous cells but unable to eliminate the cancerous stem cells, as a result, the tumor may eventually regenerate (Housman et al., 2014).

Schematic illustration of different mechanisms adopted by cancerous cells during chemoresistance is represented in Fig. 28.5.

A diagrammatic illustration of mechanisms involved in chemotherapy resistance, including enhanced DNA repair, and mutations in cell membrane receptors is shown in Fig. 28.5.

These factors can guard the cancerous cells from damage of chemotherapy drugs by making them less vulnerable (Fig. 28.5). Chemoresistance poses a substantial obstacle to the treatment of cancer since it reduces the efficacy of medication therapy. Researchers and doctors are engaged in current research in this field and are trying their best to overcome this challenge by development of new drugs, using combination therapies and development of immunotherapies. They are trying to develop new drugs with multitargeted sites and adopting new strategies. This will improve the patient's outcome. It is important to note that chemoresistance is a complex and multifactorial process, and different mechanisms can operate simultaneously or in combination. Scientists are exploring various strategies to enhance the effectiveness of chemotherapy and develop alternative treatment approaches to combat drug-resistant cancer cells to overcome the chemotherapeutic resistance.

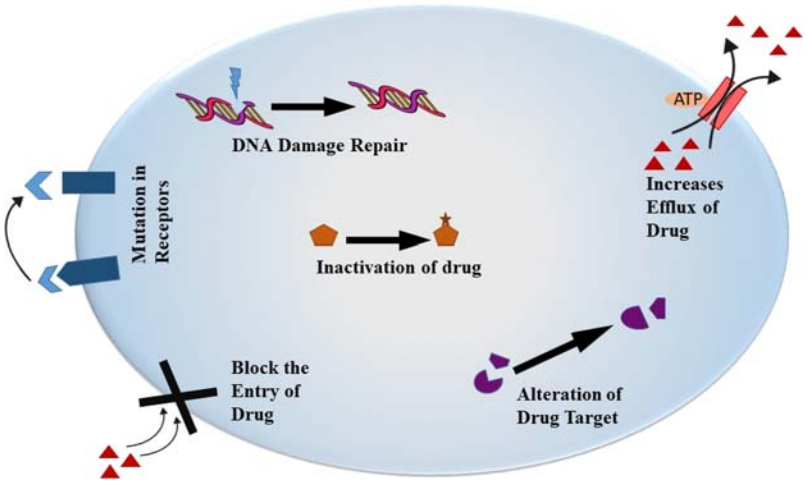

FIGURE 28.5 Diagrammatical explanation of how various mechanisms are involved in chemoresistance. This figure shows DNA repair mechanism, how the flux of drug increased, and drugs are expelled out of the cell. The figure shows that how the drug target is altered, and how uptake of drug is decreased in the cell by causing mutation in the cell membrane receptors.

3.7 Multiple drug resistance

MDR is a type of acquired resistance (shown by cancer cells or microorganisms) to chemotherapeutic drugs. It may result in overexpression of many proteins in the resistant cells. In the treatment of cancer, it is a major cause of deaths per year. In this phenomenon, cancer cells, bacteria, and viruses show resistance against effective drugs. This becomes challenging to treat that disease with this condition. This is caused due to overuse or misuse of drugs. If we are not appropriately using the drugs, then this is harmful because our cells could become resistant.

4. Conclusion and future perspectives

Cancer refers to a set of diseases that are categorized by uninhibited cell growth, and as a result, many cells are produced. Cancer may be benign or malignant. Proteases have a very important participation in our normal physiological functioning, but if these are unregulated or uncontrolled, they can cause severe damage and the normal functioning of the body. Certain proteases are involved in cancer proliferation, majorly cysteine proteases, serine proteases, and MMP. Due to their activity in diseases, it has become an active area of research, and researchers and clinicians are locating their inhibitors. We are successfully having some inhibitors that are used against cancer as drugs. However, the research is still going on to find out more perfect results. Chemotherapy is used against cancer treatment, but often cells can produce resistance against the drugs in many ways and lower the effect of drugs. The basic cause of chemoresistance or drug resistance could be the overuse and

misuse of drugs. It is due to the lack of education. People are using drugs without proper information and guidance. As the ratio of chemoresistance increases at the present speed, it would be dangerous. It will be difficult to treat even a minor disease. It will cause a burden on hospitals due to long stays, and the mortality rate will also increase. We should spread awareness about it so that we can handle the situation in future. We should start surveys to meet people to spread awareness regarding the harms of consequences. There is also a need for the development of new drugs with better efficiency. To overcome this problem, we are using different approaches such as drugs having multiple targets, immunotherapies, combination therapies, and adopting new strategies.

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CHAPTER

29

Breaking barriers: Proteases as key players in cancer chemoresistance

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1. Introduction

In the year 1884, the term protease was coined by the Dutch chemist Gerhard John Mulder from French protein. Enzymes that take part in the protein bond hydrolysis by targeting the peptide bond of the carbonyl group or the degradation of peptides that contain amino acids from proteins are called proteases or proteolytic enzymes. In humans, about 990 of protease genes are known to be present. Numerous peptides function in the development of cancer is known as a cancer degradome. Proteases are secreted in extracellular environment called as extracellular protease, which hydrolyzes the peptide bond called as endopeptidases and exopeptidases. Endopeptidases are threonine, aspartate, metalloproteinases, and serine peptidases. Based on the mechanism of bond cleavage and nature of the amino acid active sites, proteases are classified broadly into six groups.

- Cystine
- Serine
- Threonine
- Glutamic acid
- Aspartate
- Matrix metalloproteases

Proteases play critical roles at the cellular and subcellular levels such as gene expression, cell differentiation, and cell death progression; thereby it forms the blood vessels inside in the

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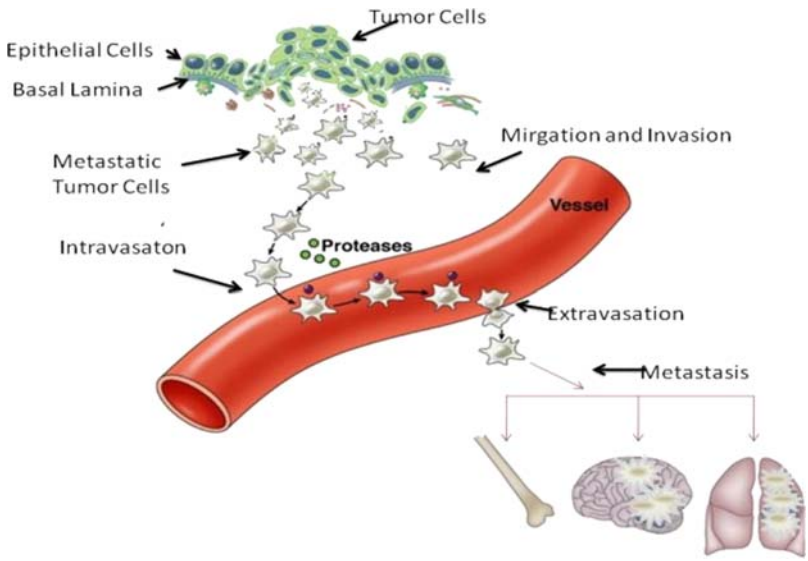

FIGURE 29.1 Entry of proteases. Proteases play critical roles at the cellular and subcellular levels such as gene expression, cell differentiation, and cell death progression; thereby it forms the blood vessels inside in the cell (angiogenesis).

cell (angiogenesis) as shown in Fig. 29.1. Subsequent migration and invasion of tumor cells into the circulatory system involve proteases, which may play a direct or indirect role in facilitating the spread to other sites. These proteases can be classified into five distinct classes. Epithelial–mesenchymal transition (EMT), which is responsible for tumor invasion, metastasis, and dissemination, increases the cell motility (Chintamaneni et al., 2022; Ren et al., 2023; Singh et al., 2017; Szebeni et al., 2017).

Proteases are key players in cellular processes such as gene expression, cell differentiation, and cell death. They also facilitate the formation of blood vessels within cells, a process known as angiogenesis.

1.1 Cysteine proteases

Cathepsins can function at both the extracellular and intracellular levels. Cathepsins are potential targets for anticancer therapy, which have extracellular activity promoting cancer cells spread to the nearby tissues before metastasizing to distant tissues. When a tumor grows, invades, and spreads, cathepsin B is actually engaged in remodeling and dissolving connective tissue and the basement membrane. This can lead to extracellular matrix degradation of the extracellular matrix by podosomes and spreading by secreted lysosomes. These cathepsins are useful prognosis markers for detection of colorectal cancer patients.

In healthy tissues and cells, a cysteine protease inhibitor called cystatin can control the activity of cathepsins. Cystatins are a class of competitive, reversible inhibitors of cysteine peptidases. In experimental trials, current research has demonstrated that cystatins can prevent the invasion or metastasis of many malignancies. The unregulated proteolysis and development of tongue cancer may be influenced by the different concentrations (Nielsen and Schmid, 2017; Singh et al., 2017; Szebeni et al., 2017).

1.2 Serine proteases

Urokinase–serine proteases known as plasminogen activators have been linked to tumor spreading, and some studies have found that the expression of these enzymes and the control of their activity are strongly correlated with the phenotype of tumors.

Matriptase, a type-2 transmembrane an enzyme called serine, is implicated in the development of certain epithelial malignancies, angiogenesis, and the breakdown of extracellular matrix. HAI-1 (hepatocyte growth factor activator inhibitor) inhibits martriptase in healthy cells. Expression of matriptase and HAI-1 loss were seen with the course of human prostate cancer (CaP). CaP is a prospective biomarker for development and a possible biomarker for determining the success of treatments.

1.2.1 Kallikerins

Recent research data implicates kallikerins in several cancer-related processes, including cell growth control, angiogenesis, invasion, and metastasis. Kallikerins are a family of S1 trypsin-like serine proteases with different expression patterns and physiological activities.

1.2.2 Trypsin

It is a well-characterized serine proteases; it plays an essential role for physiological processes (e.g., control of blood pressure, fibrinolysis, blood coagulation, food digestion).

Actually, trypsin is secreted as inactive zymogen called as trypsinogen in pancreatic juice, which is converted into active form called trypsin by the enteropeptidases in duodenal lumen. PSTI is an antiprotease mediator that protects from premature activity. PSTI is excreated through mucosa of GIT where it protects the cells from proteolytic damage. Any imbalance in protease–antiprotease system develops pancreatitis supposed to increase risk of developing pancreatic adenocarcinoma. Trypsin promotes cell proliferation, invasion, and metastasis and is also implicated in the development of colorectal cancer. Matrix metalloproteases, which are important for facilitating invasion and metastasis, are activated by trypsin and coexpressed with it. Trypsin and metalloproteases-2, -7, -9 are coexpressed and are in charge of invasion, progression, and proliferation. Adenomas are mostly converted to carcinomas by metalloproteases, which initiates invasion and metastasis. Proliferation, invasion, and metastasis are promoted by the autocrine action of trypsin and PAR-2 through a variety of mechanisms where prostaglandin production took place (Onganer, 2020; Rudzińska et al., 2019; Ryazhenov and Gorokhova, 2015; Ryazhenov et al., 2013).

1.3 Aspartate proteases

Aspartate proteases can be classified as a class of enzymes with two lobes divided by a cavity that houses the catalytic site, which is composed of two aspartate residues. The aspartic endoprotease known as cathepsin-D, or cath-D, is extensively dispersed throughout lysosomes and is in charge of the breakdown of proteins at an acidic pH as well as the breakdown of enzymes in phagasomes and lysosomes.

Procathepsin D (pCD), a mitogen that is released by cancer cells, increases the proinvasive and prometastatic features of stromal cells as well as cancer cells. Inhibiting pCD secretion from cancer cells can stop the growth of cancer cells both in vitro and in vivo, according

to certain research that shows how pCD produced from cancer cells influences various phases of tumor formation (Bowler et al., 1998; Kim et al., 2020; Pontious et al., 2019; Pranjol et al., 2019).

1.4 Threonine proteases

Threonine proteases, also known as proteasomes, play a crucial role in the destruction of cellular proteins that have been marked for degradation through a complicated process known as polyubiquitination. A technique called polyubiquitination involves adding several ubiquitin molecules to a protein that is intended to be degraded. The pathophysiology of several malignancies, including cancer, appears to be linked to aberrant proteasome-dependent proteolysis. Pharmacological proteasome function inhibition has been proposed as a potential class of anticancer medications. When the proteasome is inhibited, proapoptotic proteins accumulate in tumor-causing cells but not in normal organs (Folgueras et al., 2004; Wu et al., 2021; Yeldag et al., 2018; Zarredar et al., 2018).

1.5 Matrix metalloproteases

A highly homologous Zn^{+2} of nine or more endopeptidases called matrix metalloproteases (MMPs) cleaves the majority, if not all, of the extracellular matrix's components. Individual protease expression levels and the variety of expressed proteases both rise throughout disease states. Multiple tumor forms have increased matrix metalloprotease expression, and most often, increase correlates with reduced survival (Eatemadi et al., 2017; Masood et al., 2022; Ramos et al., 2021; Vandercappellen et al., 2008; Wojtukiewicz et al., 2015).

Extracellular matrix proteins are transformed and replaced by matrix metalloprotein.The fibrillar collagens of bone, skin, and interstices as well as nonfibrillar collagens, such as lamina, are substrates for the enzymes. Metalloprotease activity is tightly regulated, as one might anticipate from an enzyme with such potential for degradation. When MMPs are controlled by regulating gene expression, they are secreted as latent proenzymes that need to have their 10-kDa amino terminal domain removed or modified to manifest enzyme activity and exhibit enhanced expression linked to the pathophysiology of cancer. MMPs can be stopped once they have been activated by protease inhibitors such as alpha2-macroglobulin or MMP inhibitors. Enzyme control that is excessively tight might result in extracellular remodeling that is substantial, such morphogenesis or wound healing.

There are over 500 genes in the human genome that codes for proteins that are known as "proteases" or "protease-like proteins," which provides evidence of the metalloprotease-operating proteolytic system in human tissues. However, all proteolytic enzymes are linked to tumor invasion through the cleavage of basement membrane and ECM by MMPs, which enables cancer cells to pass through and enter the stromal matrix (Lin and Karin, 2007).

Studies first linked these enzymes to the ability of cancer cells to invade because of their capacity to break down every significant protein complex found in the extracellular matrix and basement membrane. Other studies have suggested that MMPs play a part in the early development of tumors by stimulating angiogenesis and promoting cell growth. MMPs stimulate the spreading of blood vessels and lymphatic tubules by cancerous cells to facilitate

TABLE 29.1 Classes and characteristics of proteases.

Properties	Cysteine	Serine	Aspartic	Mettallo/Zinc
Active site	Cysteine	Serine	Aspartic	Zinc
Location	Lysosomes	Intracellular, extracellular	Lysosomes	Intracellular, extracellular
Optimum pH	7–9	3–7	2–4	5–9
Examples	Cathepsins-B, C, H, L	Trypsin	Cathepsins-D, E, pepsinogen-1,2, urokinase-type plasminogen activator	Collagenase

local cancer growth by disrupting normal tissue. The expression of MMP is subject to spatial and temporal variations due to the presence of cytokines, growth factors, and oncogenetic products. TNF-alpha, IL-1, and other cytokines are commonly involved in the induction of MMP genes in various malignant tumors. Furthermore, the transcription of MMP is inhibited by transforming growth factors-beta or by retinoids.

Matrix metalloprotease inhibitors (MMPIs) can inhibit the cleavage of ECM in the area of proteolysis and inhibit tumor growth and metastasis. In various studies, tissue inhibitors of metalloproteinases block the MMP activity in cancer and inhibit tumor growth, but using of tissue inhibitors of metalloproteinases has technical difficulties as it happens with other macromolecules, which results in developing the synthetic type. The generation of synthetic inhibitors consisted of pseudopeptides that mimicked the cleavage sites of the substrates of MMP. This MMPI is based on the hydroxamate molecule, BB-94. In clinically, intraperitoneally administration of batimastat does not show significant response. So, it is replaced with another peptidomimetic MMPI through oral availability. Marimastat inhibits MMPs-1, 2, 3, 7, 9, 12, 13, and it is effective in pancreatic carcinoma (Eatemadi et al., 2017; Folgueras et al., 2004; Yeldag et al., 2018; Zarredar et al., 2018) (Table 29.1).

2. Role of proteases in various types of cancer

2.1 Serine proteases

2.1.1 HtrA1

The upregulation of HtrA1 expression has the potential to greatly reduce cell division, impede cell growth, and trigger apoptosis in cells. Therefore, modifying the HtrA1 molecule may be a useful therapy approach for ESCC. a negative relationship between TGFβ1 and HtrA1 expression, as well as HtrA1's degradation of TGFβ1. As everyone is aware, the TGF-β signal is a crucial signal transduction pathway inside cells. It may encourage the invasion and spread of malignant cells. Therefore, by downregulating the TGFβ1 signal, the increased HtrA1 expression may prevent carcinogenesis. According to all of these studies, HtrA1 may be able to stop the proliferation of cancer cells (Zarredar et al., 2018).

2.1.2 HtrA2

HtrA2 is overexpressed in many types of cancer and serves as a marker that promotes tutor growth. Compared with normal tissues and benign tumors, thyroid malignant tumors have higher expression levels of HtrA2. Studies revealed that hepatocellular carcinoma (HCC) patients had higher expression levels of HtrA2, and that HtrA2 may act as an oncogene. Furthermore, in HCC, a negative expression association was discovered between HtrA2 and HIF-1α. HtrA2's suppressive effects on cancer were also noted. According to Mao et al., the absence of HtrA2 caused A549 cells to express less apoptosis-related protein. The low expression of HtrA2 was linked to clinical stage and histological differentiation (Eatemadi et al., 2017).

2.1.3 HtrA3

HtrA3 is downregulated in all cases of the ovarian cancer, regardless of the tumor stage or subtype. Epithelial ovarian carcinoma had the lowest amount of HtrA3. HtrA3 expression was observed to be downregulated in breast cancer, particularly in individuals with lymph node metastases. In addition, BC without lymphatic metastases had reduced HtrA3 expression due to ER- and PR-positive status. However, when metastasis happened, there was no discernible variation in HtrA3 levels between the tumors (Masood et al., 2022).

2.1.4 HtrA4

Preeclampsia prognosis may be predicted using HtrA4, which has been shown to be important for embryo implantation and placentation and to rise markedly in the early stages of the condition. Despite this, not a lot of study has been done regarding this topic in cancer. According to the elimination of N-terminal, variations increased the chemotherapeutic impact in cancer cells. The effectiveness was determined by HtrA4's proteolytic activity, and HtrA4's N-terminal deletion is more effective at causing cell death. The process by which HtrA4 causes cell death involves the breakdown of antiapoptotic XIAP, indicating that it is connected to the apoptotic pathway. These results provide further understanding of the role of the HtrA4 protease in carcinogenesis and cell death, and they may suggest a possible therapeutic target for the treatment of cancer (Table 29.2).

TABLE 29.2 Different types of serine proteases.

HtrA proteins	Cancer type
HtrA1	Breast, bladder, pancreatic, gastric cancer
HtrA2	Thyroid, hepatocellular, colon cancer
HtrA3	Ovarian, endometrial, breast cancer
HtrA4	Lung and breast cancer

2.1.5 Trypsin role in ovarian cancer

Trypsin may generate tumors in ovarian cancer through its receptor PAR2, which is then enhanced by HE4. It is well established that the ERK signaling cascade contributes to cancer cell survival, proliferation, and treatment resistance. PAR2-mediated cell growth needed ERK phosphorylation. Additionally, some studies investigation revealed that in ovarian cancer cell lines, PAR2 activation promotes ERK activation and enhances cell proliferation (Vandercappellen et al., 2008).

2.2 Cysteine protease

2.2.1 Cathepsin B role in breast cancer

Several investigations have demonstrated a correlation between invasive and metastatic cancer characteristics and improper control of cathepsin B overexpression. Although cathepsin B is often linked to lysosomes that are involved in immunological response and autophagy, respectively, cancer has been demonstrated to result from abnormal production of this protein and mainly causes breast cancer. The progression and invasion of cancer have been linked to cysteine protease cathepsin B (CTSB). Furthermore, there was a strong positive link found in breast cancer patients' CTSB expression and positive metastatic lymph node count (Lin and Karin, 2007).

2.2.2 Cathepsin L role in pancreatic cancer

A lysosomal cysteine protease called cathepsin L breaks down extracellular matrix when cancer cells invade. In pancreatic tumor tissue, it has been demonstrated to have predictive significance (Eatemadi et al., 2017).

2.3 Aspartate protease

2.3.1 Cathepsin D role in breast cancer and ovarian cancer

It has been demonstrated that overexpression of CathD causes tumor invasion into neighboring tissue and is a poor prognostic indicator in individuals with breast cancer. According to studies, MCF7 breast cancer cells treated with estrogenic overexpress and secrete a high amount of pCathD (Yeldag et al., 2018).

2.3.2 Cathepsin E

Intracellular, hydrolytic aspartic protease known as cathepsin E (CTSE) has been shown to be expressed in erythrocytes, lymphoid tissues, immunological and gastrointestinal system cells, and cancer cells. The expression of CTSE in erythrocytes was linked to their localization at the plasma membrane and was triggered by the solubilization of the enzyme caused by cellular aging. Upon activation, CTSE started to degrade plasma membrane proteins as a component of mechanisms produced by senescence or injury to red blood cells. Furthermore, atopic dermatitis (AD)−related animals or patients' erythrocytes displayed decreased CTSE expression, indicating that an oversight in CTSE synthesis could cause AD (Folgueras et al., 2004).

3. Chemoresistance

Malignancies can acquire resistance to established therapy, and prevalence of these drug-resistant malignancies is expanding. Mechanisms of drug resistance encompass alterations at the target site and processes such as epithelial-mesenchymal transition (Dasari et al., 2024). The majority of relapses and poor patient survival outcomes are caused by chemotherapeutic drug resistance, which persists despite innovations in cancer Drugs like cyclophosphamide, nitrogen mustard, gemcitabine, methotrexate, bleomycin, carboplatin, vinblastine, paclitaxel, oxaliplatin, and satraplatin are often affected by this resistance. Multidrug resistance (MDR) refers to resistance that is limited to a single drug or to several medicines with distinct mechanisms of action (Zarredar et al., 2018) (Table 29.3).

3.1 Types of chemoresistance

Drug resistance is of two types based on the time of development. They are intrinsic resistance and acquired resistance. Initially, chemoresistance helps reduce the toxic effects and optimize the therapy (Ramos et al., 2021).

3.2 Intrinsic chemoresistance

Intrinsic chemoresistance was established before the drug was given to the patient. Resistance phenotypes were observed when tumors exhibited intrinsic resistance even before the administration of chemotherapy drugs. The variables of this resistance are various and stated as follows.

3.2.1 BRCA mutations in breast and ovarian cancers

Tumors with mutations in the BRCA genes often exhibit intrinsic resistance to certain chemotherapy drugs, such as platinum-based agents.

3.2.2 P-glycoprotein overexpression

P-glycoprotein is a membrane transporter protein that actively pumps chemotherapy drugs out of cancer cells, reducing their intracellular concentration and efficacy. Overexpression of P-glycoprotein is associated with intrinsic resistance to multiple chemotherapy drugs, including anthracyclines and taxanes.

TABLE 29.3 Different types of cancers and their drug resistance.

Cancer	Drug resistance
Breast cancer	Doxorubicin, paclitaxel, 5-fluorouracil, cyclophosphamide, and carboplatin
Gastric cancer	Cisplatin, docetaxel, 5-fluorouracil
Colorectal cancer	Oxaliplatin
Lung cancer	Paclitaxel

3.2.3 TP53 mutations

TP53, also known as the tumor protein p53, is a tumor suppressor gene that plays a crucial role in regulating cell cycle progression and DNA repair. Mutations in TP53 can confer intrinsic resistance to chemotherapy by promoting cell survival and evasion of apoptosis.

3.2.4 Increased DNA repair capacity

Cancer cells with enhanced DNA repair mechanisms can efficiently repair chemotherapy-induced DNA damage, leading to intrinsic resistance to DNA-damaging agents such as alkylating agents and platinum drugs.

3.2.5 Activation of antiapoptotic pathways

Dysregulation of apoptotic pathways, such as the Bcl-2 family of proteins, can protect cancer cells from undergoing apoptosis in response to chemotherapy-induced stress, contributing to intrinsic resistance.

3.2.6 Altered drug metabolism

Changes in drug metabolism pathways, including drug activation, detoxification, and efflux, can result in reduced intracellular drug concentrations and intrinsic resistance to chemotherapy.

3.2.7 Microenvironment-mediated resistance

Tumor microenvironment factors, such as hypoxia and stromal interactions, can promote intrinsic chemoresistance by limiting drug delivery to cancer cells, promoting cancer cell survival, and inducing drug resistance pathways.

3.3 Inherent genetic mutations in tumors

A transcription factor called NFE2L2 controls the transcription of genes involved in drug efflux, detoxification, growth factors, and antioxidants. KEAP1 controls the activity of NFE2L2. Mutations impact these two genes. NFE2L2 overexpression or their interaction of cancer tissues, including the liver, ovary, bladder, breast, and lung. Research on nonsmall lymphocytic pulmonary cancer in animals shows to what degree the KEAP1 deletion and NFE2L2 mutations assist in the expansion and survival of cancer cells. Chemoresistance is induced by KEAP1 deletion in preclinical models of lung adenocarcinomas and lung squamous cell carcinomas.

3.4 Heterogeneity of tumor cell population

This cell subpopulation is more prevalent in several different malignancies after treatment. As an illustration, breast cancer stem cells (BCSCs) exhibit traits such as self-renewal, differentiation, metabolism, drug efflux transporter overexpression, DNA repair, immune evasion, etc.

3.5 Pharmacological factors

Clinical resistance can be caused by a variety of things, including insufficient concentration of drug at the tumor, difficulties in achieving optimal pharmacokinetic profile of the medication, the alteration of the drug target, the alteration of drug uptake, distribution, metabolism, or elimination. Taxanes and anthracyclines are used to treat breast cancer. Taxanes attach to beta-tubulin and promote mitotic arrest and cause apoptosis in breast cancer cells (Yeldag et al., 2018). Thymosin 4 (T4), a protein that binds to G-actin monomers, was enhanced in the drug-resistant cells by demethylating the DNA, and the promoter region undergoes active histone H3 modification. The HCC cell line acquired stem cell by overexpression of T4, which also caused resistance to the vascular drug sorafenib.

4. Mechanism of chemoresistance in cancer

There are four stages in which a chemotherapeutic medication exerts. Prior to being activated, the medication first enters the cell. The medicine works inside the cell to affect its target(s), which ultimately causes cell death. At any of these four phases, chemoresistance can emerge (Wu et al., 2021) (See Fig. 29.2).

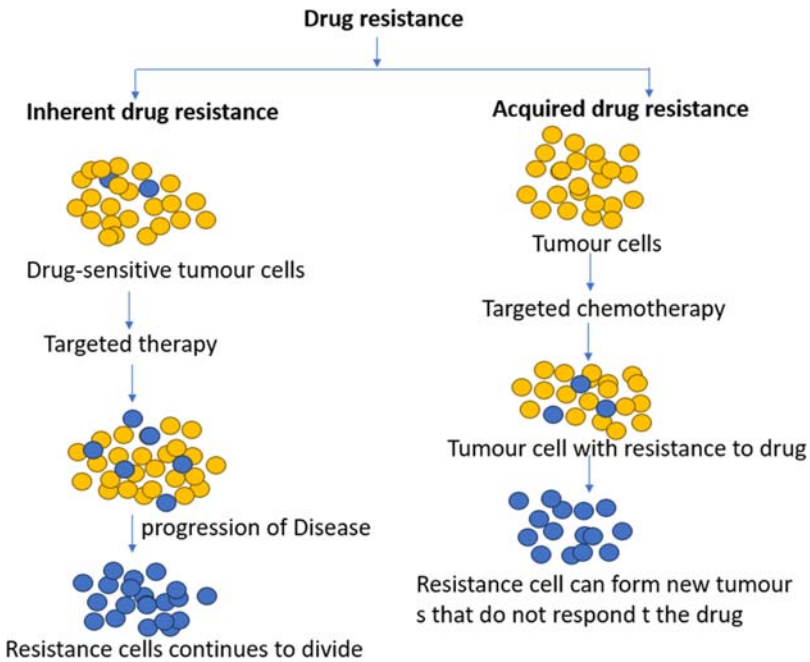

FIGURE 29.2 Diagrammatic representation of types of chemoresistance. Inherent drug resistance, represented in the diagram, depicts preexisting factors that render cells less susceptible to chemotherapy drugs. Acquired drug resistance, also illustrated, develops over time as cells adapt to the effects of treatment through mechanisms such as enhanced DNA repair and increased drug efflux. The diagram visually distinguishes between these two types of chemoresistance and their respective underlying mechanisms.

The diagram visually outlines chemoresistance, with one section showing inherent resistance based on existing cellular factors, while another depicts acquired resistance arising from prolonged exposure to chemotherapy. Each segment illustrates that different ways cells evade drug effects, such as enhanced DNA repair or increased drug expulsion.

4.1 Drug influx–efflux

Chemotherapeutic medicines need to get into the cells with optimal intracellular concentration for them to be effective. Drug influx is affected by a number of variables, including the presence and type of influx pumps used to transport the drug, the manner in which the drug is disseminated, the extent of the drug's absorption, the extent to which the tumor is expanding, the physical properties of the site of the tumor, the structural and functional characteristics of the vasculature in the tumor, the degree to which the tumor is necrotized and the mechanisms by which the drug is transported through the microvessel wall and in the extracellular space (Yeldag et al., 2018).

A class of membrane-bound proteins known as solute carrier (SLC) transporters act as I pump of influx to move the substrates across membranes of the cell. They are crucial to biological activities ranging from cellular absorption of minerals. Decreased drug binding to the transporters and decreased transporter density are two possible causes of decreased drug influx into the cell. The medications pralatrexate and methotrexate are helped traverse the cell membrane via the SLC transporter glycoprotein-reduced folate carrier (RFC). Methotrexate's propensity to bind the transporter is decreased. In osteosarcoma and myeloma, decreased RFC expressions result in methotrexate resistance (Folgueras et al., 2004).

The drug's therapeutically effective concentration is also prevented by enhanced efflux. The control of chemical substance distribution, absorption, and excretion is carried out by ABC proteins found in the cell membrane. These proteins prevent medications from entering cancer cells, reduce the drugs bioavailability, and preserve the drugs intracellular concentration (Ryazhenov and Gorokhova, 2015) (Table 29.4).

TABLE 29.4 ABC protein overexpression.

ABC protein	Overexpressed in cancers	Drugs effluxes
(P-gp, ABCB1)	Lung, liver, kidney, breast, ovary	Doxorubicin, epipodophyllotoxins, actinomycin D, methotrexate, and mitoxantrone, paclitaxel
(BCRP/ABCG2)	Breast, prostate, colon, stomach	Cisplatin, anthracyclines, irinotecan, methotrexate
(MRP1/ABCC1 and MRP2/ABCC2)	Neuroblastoma	Etoposide, methotrexate, vincristine, vinblastine

4.2 Epithelial–mesenchymal transition

Epithelial cells change into stromal mesenchymal cells through a process known as EMT. They become more migratory and invasive, losing the adhesion of cell, and encouraging metastasis. Cancer metastasis, progression, and medication resistance to treatment are all influenced by EMT. EMT can be triggered by a number of factors, Drug resistance is influenced by EMT-inducing transcriptional factors (EMT-TFs). Twist, Snail, Slug, and Zinc Finger EMT-TF overexpression ZEB1 and drug resistance in breast cancer are known to be induced by Forkhead box C2 (FOXC2). ZEB1 triggers EMT by reducing epithelial microRNAs such as the members of the miRNA-200 family, which reduce the epithelial phenotype. By boosting medication efflux by ABC transporters, several EMT-TFs encourage resistance. Paclitaxel-induced cell death is reduced by FOXM1 or ABCC5 downregulation, which also decreases drug efflux (Ramos et al., 2021).

4.3 Drug activation and inactivation

Through the direct mechanism of cancer medicines or by inhibiting the pathway, GST enzymes cause treatment resistance. Cytochrome P450 (CYP) system inactivation of drugs is a different method. It is hypothesized that mutations in these enzymes would cause the medications to be broken down and secreted, lowering their optimal concentration and resulting in drug resistance. Irinotecan, a topoisomerase I inhibitor used in the treatment of colon cancer has been demonstrated to be inactivated by CYP450 class I metabolizing enzymes. Bleomycin hydrolase inactivates the antibiotic bleomycin, a DNA-binding glycopeptide. High amounts of this enzyme are seen in bleomycin-resistant malignancies, whereas low levels are found in sensitive tumors (germ cell cancers, lymphomas, and squamous carcinomas) (Eatemadi et al., 2017).

4.4 Alteration of drug targets

Numerous anticancer medications work by interacting with intracellular target proteins to impose their cell-damaging effects. Any change in these target enzymes' expression levels or functions eventually affects how well medicines work. It is significant to note that dysregulation of the enzymes has been demonstrated to result in resistance to the drugs 5-fluorouracil and tomudex, methotrexate, and doxorubicin correspondingly. Additionally, alterations in the amount of estrogen and progesterone receptor expression in individuals with breast cancer neutralize the effects of tamoxifen therapy (Masood et al., 2022).

4.5 Enhanced DNA repair and impaired apoptosis

One of the ways chemotherapeutic medications destroy cancer cells is by causing DNA damage. The cells will avoid cell death and acquire chemoresistance if they can repair the DNA damage that has been done to them. The damage brought on by drugs can be repaired through DNA damage response (DDR) mechanisms (Wojtukiewicz et al., 2015).

In a murine orthotopic model of GLL multiforme orthotopic tumor, a DDR process mediated by the upregulation induced by the Wnt signaling pathway resulting in the upregulation

of a metabolite (O(6)-methylguanine DNA methyltransferase). This process resulted in the development of resistance to temozolomide. As a result of the inhibition of the Wnt pathway, MGMT expression was reduced, and the chemosensitivity to DNA-altering agents was restored. Similarly, following chemotherapy-induced downstaging, a strong response to treatment and surgical resection were favorably linked with reduced expression of MRE11. Furthermore, palbociclib showed aberrant DNA repair activity, while therapy with PARP inhibitors, olaparib and niraparib, dramatically reduced palbociclib resistance in cancer (Pranjol et al., 2019).

Apoptosis is caused by two different pathways. One is extrinsic, which involves ligands such as FAS and TNF-Rs, as well as linker proteins such as caspases 3, 6, 7, and 8. The other is intrinsic, which is mediated by mitochondria. For example, in multiple myelocytic leukemia, higher levels of BCL-2 and BCL-XL, as well as overexpressing caspase 3, have been shown to inhibit cell death (Bowler et al., 1998).

5. Techniques for preventing chemoresistance in cancer

5.1 Early diagnosis of cancer

Detection methods include identifying DNA mutations and single nucleotide polymorphisms, profiling coding and noncoding transcripts, analyzing protein expression, and detecting circulating tumor DNA (ctDNA).

5.2 Combination therapy

Non−cross-resistant combination of chemotherapeutics drugs, using natural and small molecule.

5.3 Enhancing the drug effectiveness

Altering the dosage of anticancer medications, nanoparticle-based delivery to the tumor cells, using antibody−drug conjugates (ADCs).

5.4 Early diagnosis of chemoresistance

New tumor assays, biomarker testing, positron emission tomography (PET), high-throughput pharmacogenomics, CRISPR screening.

5.5 Targeting cancer stem cells

The study focused on the targeting of surface biomarkers, including CD133, CD44, CD24, ESAs, and CD47, as well as the targeting of proliferative pathways, stemness, and EMT signaling. Additionally, the study addressed the modulation of microenvironment signals, including angiogenesis and hypoxia, as well as acidic pH (Zarredar et al., 2018).

See Table 29.5.

See Fig. 29.3.

TABLE 29.5 Various types of cancers and its targets.

Action	Mechanism	Drugs	Type of cancer
Enhanced DNA repair	MGMT	Temozolomide	Various cancers
	Wip1, a suppressor of the ATM-dependent signaling pathway	Cisplatin	Oral squamous cell carcinoma
Signaling mechanisms	WNT/β-catenin	5-Fluorouracil	Neuroblastoma
	Notch	Cisplatin	Hepatocellular carcinoma
Efflux of the drug	ABC transporters	Actinomycin D	Most cancers
Influx of the drug	SLC transporters	Methotrexate	Multiple myeloma
Influx of the drug	pH	Cyclophosphamide	Most cancers

ABC, ATP-binding cassette; *ATM*, ataxia telangiectasia mutated; *EMT*, epithelial–mesenchymal transition; *MGMT*, O(6)-methylguanine DNA methyltransferase; *SLC*, solute carrier.

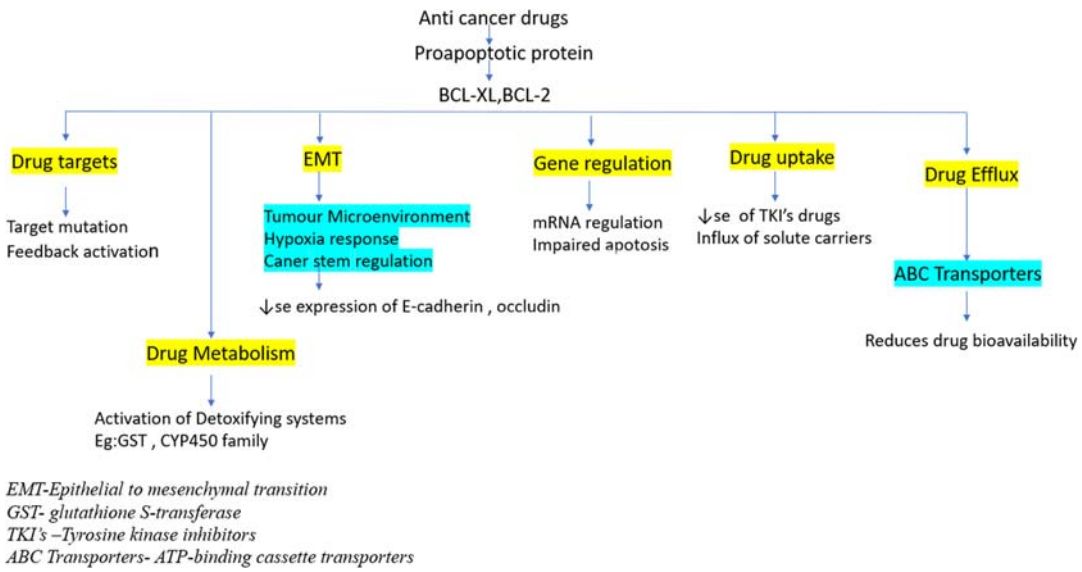

FIGURE 29.3 Mechanism of chemoresistance. This figure illustrates various mechanisms of chemoresistance: drug targets, drug metabolism, epithelial-to-mesenchymal transition (EMT), gene regulation, drug uptake, and drug efflux. Each pathway represents distinct ways cancer cells can become resistant to chemotherapy drugs, such as altering drug targets, enhancing metabolism, or reducing drug uptake while increasing efflux.

This diagram shows different routes through which cancer cells resist chemotherapy, including changes in drug targets, metabolism, gene regulation, uptake, and efflux. Each pathway represents that unique strategy cells employ to evade the effects of chemotherapy drugs.

6. Conclusion

When cancer progressed, the patient occasionally faced an accelerated mortality from the anticancer drugs or chemotherapy they were receiving since the cancer cells have resistance to the drugs occasionally resulted in toxicity. Here proteases are mostly responsible for cancer, and further studies are also carried out on the proteases with respect to signaling pathways. Chemoresistance is most commonly seen in cancer patients due to TME, impaired apoptosis, DNA alteration, drug metabolism, and drug activation and inactivation.

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SECTION IV

Other aspects

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CHAPTER

30

Matrix metalloproteases—targeting low-molecular-weight compounds

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Abbreviations

ApoE apolipoprotein E
BBB blood—brain barrier
EGFR epidermal growth factor receptor
LDL low-density lipoprotein
LPS lipopolysaccharide
LRP1 LDL receptor-related protein 1
MMPs matrix metalloproteinases
NMDAR *N*-methyl-D-aspartate receptor
NNGH *N*-isobutyl-N-[4-methoxyphenylsulfonyl]glycyl hydroxamic acid
OA osteoarthritis
PDF Fe-peptide deformylase
SDS sodium dodecyl sulfate
TACE TNF-α-converting enzyme
TAPI-1 TNF-α-processing inhibitor-1
TNF-α tumor necrosis factor-α
α2M α2-macroglobulin
α2MR α2M receptor

1. Current human MMP therapeutics under clinical trials

From direct association with MMPs enzymes in initiation and progression of cancer, immune, brain and vascular diseases, the MMP inhibitors are considered as potential

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therapeutics. Among MMPs, limited MMP class including MMP-2, -9, -13, and MT1-MMP have been known not to be related to a side effect of musculoskeletal syndrome frequently occurred with broad-spectrum MMP inhibitors. Especially, MMP-9 inhibitors can protect ischemic brain damages, epilepsy, hypoglycemia, stroke, and traumatic brain injury. MMPs inhibitors such as BB-94 (batimastat) or GM600 exhibited reliable positive efficacies in the acute animal stroke models, although their blood—brain barrier (BBB) permeability is quite limited (Rempe et al., 2016). Similarly, carnosine as an effective zinc-chelating agent diminishes the levels of ischemic injury in animal stroke models via breakdown inhibition of BBB, as administered by intravenous injection. Carnosine inhibits tight-junction proteins damage caused by the endothelial MMP-2/9 activities (Kim et al., 2021). Similarly, tetracycline derivatives such as doxycycline and minocycline inhibit MMP-2/-9 in a rat ischemic animal model (Machado et al., 2006). Furthermore, minocycline administration to human ischemic stroke patients was reported that it is not toxic in preceded clinical trials (Fagan et al., 2010). In zinc neuronal toxicity protection effect, neuronal cells in ischemic neuronal injury toxic model are zinc toxicity—induced and the cells-released lactate dehydrogenase levels are measured to calculate the cell death. Finally, drug itself evaluation for neuronal toxicity and antiinflammatory inhibition are prerequisite.

Currently, the only MMP inhibitor approved by US FDA is the periodontal disease-oriented periostat, which active ingredient is doxycycline hyclate (Table 30.1). Neuronal cell death—based stroke shares the similar mechanistic progression with the traumatic brain injury, epilepsy, and amyotrophic lateral sclerosis. Therefore, MMP-9 inhibitors that can protect ischemic brain damages, epilepsy, hypoglycemia, stroke, and traumatic brain injury can be similarly approached to protect the neuronal degeneration. For outstanding merit of low-molecular-weight MMP inhibitors, they can be applied for prevention of human diseases such as cancer and ischemic injuries.

2. Light molecular weight MMP inhibitors are especially candidates for ischemic stroke disorders

Zinc neurotoxicity has been targeted for therapeutic agents of complexed neuronal cell death. Zinc-dependent MMP inhibition has been known to inhibit ischemic brain injury, and thus, MMP inhibitors can be used for stroke treatment drugs. Preexisting MMP inhibitors such as broad-spectrum inhibitors of GM 6001 and JNJ-0966 or selective MMP2/9 inhibitor SB-3CT have been suggested to be effective for traumatic brain injury. Similarly, a selective MMP-9 inhibitor minocycline protects neurodegenerative progression in animal studies. However, the clinical trials are failed due to lack of MMP selectivity or specificity, inhibiting all the MMP types. Thus, selective and specific MMP subtype inhibitors are required without toxicity. Neuropsychiatric disorder—related MMP-9-specific inhibitors that are not targeting for active site but inhibit MMP-9 activation process in pathological conditions are required. Because stroke disorder is the disease that occurs by death of diverse cells, drugs to control all the neurotoxic mechanism(s) are required. Several low-molecular-weight MMP inhibitors described are especially being considered as candidates for ischemic stroke disorders. In Table 30.2, the representative development status under clinical trials of human MMP therapeutics has been presented.

TABLE 30.1 The representative development status under clinical trials of human MMP therapeutics.

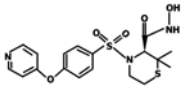
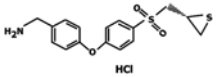
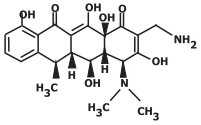
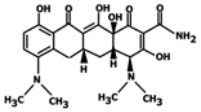
Compound family	Drugs	Potency (IC_{50})	Clinical trial status and features	Structure	Diseases
Antibody	Andecaliximab (ADX) (CAS No. 1518996-49-0)	Sub nM	Phase 2, but not effective, chemotherapy combination. Phase 2 and phase 3 completed in 2019. Anti-MMP-9 monoclonal antibody (Gilead Sciences).	Monoclonal antibody as IgG polypeptide for the **Polypeptide**	Gastrointestinal tract inflammation: gastric/ gastroesophageal junction diseases.
Hydroxamic acid	Prinomastat (CAS No. 192329-42-3)	Low nM	Phase 3, nonselective MMP inhibitor, metabolic toxicity.	Prinomastat 	Combination with gemcitabine.
Thiols and Thiirane	(R)-ND-336 (CAS No. 2252493-33-5)	19 nM (Ki) for MMP-9	Selective MMP-2/9 and MMP-14 inhibitor.	(R)-ND-336 	Covalent inhibitor, MMP-3,-9,-24-related diseases.
Tetracyclines	Periostat (doxycycline hyclate)	2–5 mM	FDA-approved sole drug, periodontal inflammatory disease. Phase 4 for multiple sclerosis.	Periostat 	Pan MMP inhibitor-applicable all disease.
	Minocycline (CAS No. 10118-90-8)	100–300 mM	Phase 4 stroke in cardiac atherosclerosis. MMP-9 inhibitor. With DME in phase I/II clinical trials. IC_{50} at 180 uM for MMP-9. Clinical trial for traumatic brain injury protection.	Minocycline 	Effective for microglial neurodegeneration. Cerebral ischemic excitotoxicity. Inhibition of caspases-1 and -3.

TABLE 30.2 The representative development status under clinical trials of human MMP therapeutics.

Drugs	Potency (IC_{50})	Clinical trial status and features	Structure	Diseases
Ilomastat (GM6001)	IC_{50} to each target MMP:1.5 nM for MMP-1; 1.1 nM for MMP-2; 1.9 nM for MMP-3; 0.5 nM for MMP-9	Broad spectral inhibition for MMP-1, -2, -3, -7, -8, -9, -12, -14, and -26 inhibitors.	Ilomastat	Endothelium intimal thickening of arteriosclerotic lesion. Prevention of corneal ulceration.
JNJ0966 (CAS 315705-75-0)	$IC_{50} = 440$ nM.	Selective pro-MMP9 activation inhibitor to active MMP9.	JNJ0966	BBB penetration. Invasion suppression. Inhibition of pro-MMP-1, -2, and -3.
SB-3CT (CAS 292605-14-2)	Each Ki value: 13.9 nM for MMP-2 and 600 nM for MMP-9.	Competitive MMP-2/-9 inhibitor.	SB-3CT	Gelatinase selectivity. BBB permeability. Neuroprotective and anticancer MMP-2/3 specificity compared with MMP-9.
GI254023X (GI4023; SRI028594) (CAS 260264-93-5)	IC_{50} value: 2.5 nM for MMP-9. 5.3 nM for ADAM10.	Inhibition for MMP-9 and ADAM10.	GI254023X	Increase in plasma Aβ40 level from the brain. Inhibition of dorsal root ganglion ADAM10-mediated neuronal outgrowth.
Actinonin (CAS 13434-13-4)	Ki (0.28 nM)	Naturally occurring antibiotic. Inhibitor of aminopeptidase M, aminopeptidase N and leucine aminopeptidase. Zn-, Ni-, and Fe-peptide deformylase (PDF) inhibitor.	Actinonin	Natural antibacterial agent of *Actinomyces*. Inhibition of CD13, MMP-1, -2, -3, -7, -8, -9, -10, -12, and -13 and heparin.
NSC 405020 (CAS 7497-07-6)	$IC_{50} > 100$ μM	Specific MMP-14 inhibitor (MT1-MMP inhibitor) as noncatalytic inhibitor.	NSC 405020	Vestibular schwannoma, hemostasis, and thrombosis
CL-82198 (CAS 307002-71-7)	$IC_{50} = 10$ μM	Selective MMP-13 inhibitor. Binding to MMP-13 entire S1′ pocket.	CL-82198	Osteoarthritis. Noninhibitory activity to MMP-1, -9, and ADAM17 (TACE).
CTS-1027 (CAS 193022-04-7)	IC_{50} value: 0.4 nM for MMP 2. 0.6 nM for MMP13	Small molecule MMP-2/-13 inhibitor. Broad-spectrum MMP inhibitor (Ki < 9 nM for MMP-2, -3, -8, -9, -12, -13, and -14).	CTS-1027	MMP-1 selectivity. Cholestatic liver injury and hepatic fibrogenesis. Orally bioavailable.
T-26c (CAS 869296-13-9)	$IC_{50} = 6.75$ pM	Selective MMP-13 inhibitor	T-26c	Collagen breakdown. Orally bioavailable

2.1 Ilomastat (GM6001)

MMP inhibitor ilomastat (named Galardin) is a broad spectral and low-molecular-weight inhibitor specific for various MMPs (IC_{50} values 1.5 nM for MMP-1; 1.1 nM for MMP-2; 1.9 nM for MMP-3; 0.5 nM for MMP-9). It shows a relatively low inhibitory Ki value of 0.4 nM for MMP-1. Ilomastat inhibits human skin fibroblast MMP-1 known as collagenase, thermolysin, elastase, gelatinase A/B, corneal ulceration, intimal hyperplasia, and collagen synthesis (Schultz et al., 1992). Ilomastat inhibits endothelium intimal thickening of arteriosclerotic lesion (Li et al., 2002). GM6001 shows MMP-9 inhibition at 0.1 nM level and hence is used for MMP-9-related signaling pathway inhibition.

2.2 JNJ0966

JNJ0966 is a pro-MMP-9 (zymogen) inhibitor that is highly selective for pro-MMP-9. It inhibits the pro-MMP-9 conversion to active MMP-9, but does not inhibit directly MMP-9 enzymatic activity. It has a selectivity for pro-MMP-9 over pro-MMP-1, -2 and -3 with BBB penetration and cell invasion suppression (Scannevin et al., 2017).

2.3 SB-3CT

SB-3CT is a competitive MMP-2/-9 inhibitor with a gelatinase selectivity. SB-3CT shows BBB permeability with neuroprotective and anticancer activities. In an animal model of traumatic brain injury, SB-3CT inhibits MMP-9 and PC3 tumor growth by direct inhibition of bone tissue ECM degradation (Lee et al., 2015). SB-3CT enhances bone integrity (Bonfil et al., 2006). SB-3CT inhibits human intraosseous PC3 cell growth in the fetal femur in SCID mice (Bonfil et al., 2006). SB-3CT is specific for MMP-2/3 rather than MMP-9 without traumatic brain injury protection effect. This, it is used only forMMP-9 study.

2.4 Minocycline

Minocycline as an antibiotic for skin infections such as acne is called Minomycin and Minotabs. Minocycline is effective for microglial and inflammatory neurodegeneration and protects against cerebral ischemic excitotoxic neuronal death (Malhotra et al., 2018). Minocycline also inhibits caspases-1 and -3 in Huntington disease model mouse (Chen et al., 2000). Oral minocycline was used to treat patients with DME in phase I/II clinical trials. Although minocycline shows high IC_{50} at 180 uM for MMP-9 inhibition, its clinical trials are under investigation for traumatic brain injury protection, indicating that low dose concentration of MMP inhibitor minocycline is not prerequisite for traumatic brain injury protection but minocycline's inhibition of exciting toxicity is important.

2.5 GI254023X (GI4023, SRI028594)

GI254023X is a specific ADAM10 and MMP-9 inhibitor. Metalloproteinase domain containing protein 10 (ADAM10) metalloprotease and α-secretase enhance the

ectodomain shedding of low density lipoprotein (LDL) receptor-related protein 1 (LRP1). LRP1 (CD91)-ligand binding modulates lipopolysaccharide (LPS)-induced inflammatory responses. CD91 ligands known include soluble nonpathogenic cellular prion protein, inactive tissue-type plasminogen activator, and α2-macroglobulin (α2M). α2M specifically binds to LRP1. α2M suppresses macrophage LRP1 shedding, LRP1 converting to a proinflammatory molecule. AD mouse model treatment with GI254023X reduces shedding capacity of brain LRP1 known as CD91. CD91 also acts as apolipoprotein E (ApoE) receptor and activated α2M receptor (α2MR), in the membrane receptor-mediated endocytosis. GI254023X enhances plasma amyloid Aβ40 level from the brain (Herzog et al., 2014).

2.6 Actinonin

Actinonin is an antibacterial molecule of *Actinomyces* species. Actinonin inhibits MMP-1, -3, -8, and -9 and heparin as well as aminopeptidase enzymes such as M, N, and leucine type. Actinonin also causes an apoptotic cell death toward cell proliferation and tumor growth inhibition (Lee et al., 2004). Actinonin is also a bacterial Zn-, Ni-, and Fe-peptide deformylase inhibitor.

2.7 NSC 405020

NSC 405020 is a specific MMP-14 inhibitor via direct interaction with the MMP-14 hemopexin domain. NSC 405020 suppresses the Notch3 expression, usable to thrombosis, hemostasis, and vestibular schwannoma formation (Ren et al., 2020).

2.8 CL-82198

CL-82198 as a selective MMP-13 inhibitor binds to the MMP-13 S1′ pocket. CL-82198 prevents osteoarthritis progression (George et al., 2017).

2.9 CTS-1027

CTS-1027 as a small molecule MMP-2/-13 inhibitor has 1000-fold selectivity over MMP1. In mouse experiments, CTS-1027 protects liver cells from the hepatic fibrogenesis, apoptotic cell death of hepatocytes, and cholestatic liver injury. CTS-1027 also prolongs the survival length of overall animals (Kahraman et al., 2009).

2.10 T-26c

T-26c is a specifically selective MMP-13 inhibitor with 2600-fold selectivity over the other metalloenzymes. T-26c inhibits the collagen breakdown in IL-1β and oncostatin M—stimulated cartilage (Nara et al., 2014).

2.11 Histatin 5 TFA

Histatin 5 TFA is MMP-2/-9 inhibitor. Histatin 5 is a low-molecular-weight salivary protein. Histatin 5 (DSHAKRHHGYKRKFHEKHHSHRGY) inhibits the host MMP-2/-9 activity. Histatin 5 is an inhibitor of mitochondrial respiration (Gusman et al., 2001).

2.12 (S,S)-TAPI-1

(S,S)-tumor-necrosis factor-α (TNF-α) processing inhibitor-1 or TNF-α protease inhibitor-1 [(S,S)-TAPI-1] is an MMP and ADAM17 inhibitor. (S,S)-TAPI-1 is a TAPI-1 isomer known as an ADAM17 as TNF-α-converting enzyme (TACE) inhibitor and MMP inhibitor (Moss and Minond, 2017). ADAM7 (TACE) in membrane helps shedding of TNF-α receptor and IL-6 receptor as well as epidermal growth factor receptor and Notch receptor ligands. ADAM7 develops articular cartilage and osteoarthritis. ADAM7 inhibitor is an OA therapeutic option (Fig. 30.1).

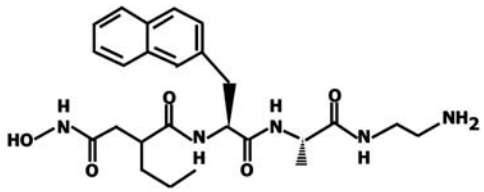


FIGURE 30.1 Chemical structure of **(S,S)-TAPI-1**.

2.13 TAPI-0

TAPI-0 as a TACE (ADAM17) inhibitor is also an MMP inhibitor and also attenuates TNF-α proteolytic activation (Balakrishnan et al., 2006). TAPI-0 inhibits thrombin-stimulated connective tissue growth factor (CTGF) expression, ERK, and c-Jun phosphorylation in ADAM17/EGFR/ERK-derived thrombin-based CTGF upregulation (Fig. 30.2).

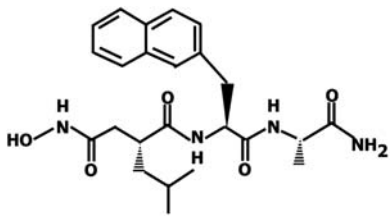


FIGURE 30.2 Chemical structure of TAPI-0.

2.14 ONO-4817

ONO-4817 as an orally active MMP inhibitor suppresses atherosclerotic rupture formation and progression caused by intimal thickening. ONO-4817 inhibits the intimal endothelial

remodeling in hyperlipidemic animal models (Okamoto et al., 2007). ONO-4817 suppresses TNF-α-driven soluble CD146 expression in human microvascular endothelial cells. ONO-4817 reduces intimal growth compared to the intima-to-media thickening ratio by MMP-2 inhibition and TIMP-2. ONO-4817 prevents neointimal growth in cardiac vasculopathy patients (Fig. 30.3).

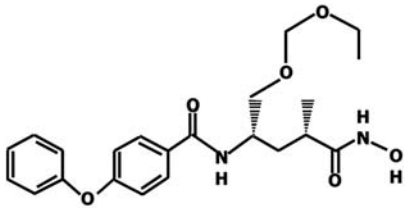


FIGURE 30.3 Chemical structure of ONNO-4817.

2.15 Quinazoline otaplimastat

N-[3-(2,4-dioxo-1,4-dihydro-2H-quinazolin-3-yl)propyl]-N-{4-[3-(2,4-dioxo-1,4-dihydro-2H-quinazolin-3-yl)propylamino]butyl}acetamide (named SP-8203) is found from earthworm as an MMP inhibitor. Quinazoline otaplimastat (SP-8203) inhibits mortality aggravation and hemorrhagic transformation during cerebral ischemic delayed thrombolysis. SP-803 competitively inhibits *N*-methyl-D-aspartate receptor (NMDAR)-induced cell excitotoxicity and protects neuropathies, improving ischemic cognitive brain impairment via NMDA receptor. Otaplimastat shows antioxidant and brain ischemic injury—protecting activity (Kim et al., 2020) (Fig. 30.4).

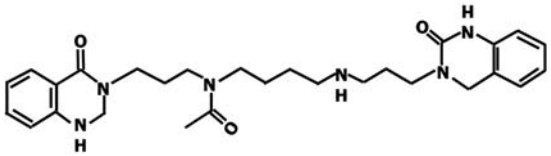


FIGURE 30.4 Chemical structure of quinazoline otaplimastat.

2.16 PF-00356231

PF-00356231 hydrochloride is a non—peptide-specific MMP-12 inhibitor that is not a zinc chelator. PF-00356231 hydrochloride forms the complex form between PF-00356231 and MMP-12. PF-00356231 inhibits MMP-3, -8, -9, and -13 (Morales et al., 2004). The PF-00356231complex structure shows that the central thiophene ring is located over the Zn atom of the S1′ pocket. An acetohydroxamate anion chelates the zinc atom. Hydrophobic interaction of PF-00356231 and S1′ pocket amino acid residues are key for complex formation. Specific selectivity of PF-00356231 for MMP-12 has been suggested over other MMPs. F-00356231 hydrochloride inhibits MMP-12/13 activity in the presence of acetohydroxamate (Fig. 30.5).

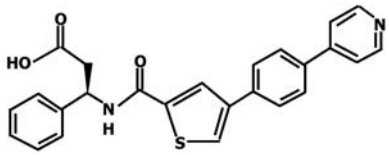


FIGURE 30.5 Chemical structure of PF-00356231.

2.17 A dual tubulin and MMP inhibitor

Dual inhibitor of tubulin and MMPs is a tubulin/MMP-IN-2. Tubulin/MMP-IN-2 specifically induces cell apoptotic death via blocking tubulin polymerization. Tubulin/MMP-IN-2 inhibits MMP-2, -3, and -9 enzymes (Huang et al., 2022). Dual inhibitor of tubulin and MMP, 9e inhibits growth of human cancer cells ($IC_{50} = 0.19-0.42$ μM) with lower cytotoxicity. 9e inhibits tubulin polymerization with apoptosis and cell cycle arrest induction at G2/M stage. It suppresses the genesis of Bcl-2 and induces Bax, p53, and caspase-3 for mitochondria-dependent apoptosis. A dual MMP and tubulin inhibitor compound 9e is promised for cancer therapy (Fig. 30.6).

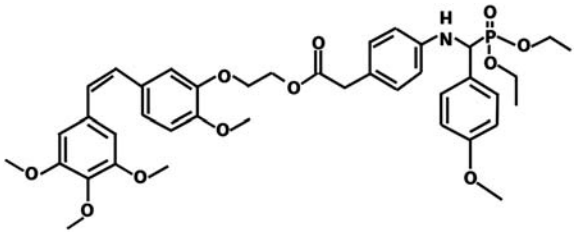


FIGURE 30.6 Chemical structure of tubulin/MMP-IN-2.

2.18 MMP13-IN-5

MMP13-IN-5 (compound 13m) is a selective MMP-2/-13 inhibitor involved in osteoarthritis (OA). It also inhibits MMP-1, -2, -9, -13 and -14 (Cuffaro et al., 2023).

Chemical structure of MMP13-IN-5 (Fig. 30.7).

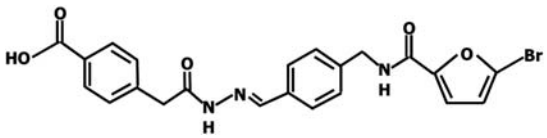


FIGURE 30.7 Chemical structure MMP13-IN-5.

2.19 MMP-2/-9-IN-1

MMP-2/-9-IN-1 is an oral and selective MMP-2/-9 type IV collagenase inhibitor. MMP-2/MMP-9-IN-1 blocks growth and metastasis of cancer cells (Tamura et al., 1998). MMP-2/-9 inhibition improves mice cognitive function (Fig. 30.8).

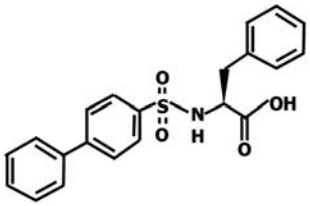


FIGURE 30.8 Chemical structure of MMP-2/-9-IN-1.

2.20 ARP101

ARP101 as a specific MMP-2 inhibitor causes cancer cell death via autophagosome formation and LC3I conversion to LC3II form (Jo et al., 2011). ARP-100 binds to MMP-2 S1′ pocket. ARP-100 also exhibits the MMP-1, -3, -7, and -9 inhibitory activities. ARP101-caused autophagosome formation is inhibited in autophagy-related gene 5 (ATG5)—negative embryo fibroblasts. ARP101 inhibits cell growth and invasion. ARP101 also activates noncanonical sequestosome 1/p62-Keap1-Nrf2 pathway. ARP101 activates LC3 II. ARP101 stabilizes Nrf2 and allows NrF2 translocation to the nucleus and transcription of antioxidant genes (Fig. 30.9).

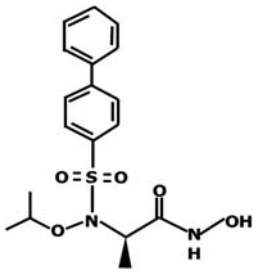


FIGURE 30.9 Chemical structure of ARP101.

2.21 Marimastat

Marimastat (BB2516) is an orally active and broad spectral MMPs inhibitor against MMP-1, -2, -7, -9, and -14 in the tumor treatment. It is also an angiogenic and metastasis inhibitor in combination with chemotherapy and radiation to inhibit tumor growth (Skipper et al., 2008). Marimastat without side effects inhibits aging-related senescence-β-galactosidase activity. Marimastat induces mTOR regulation and autophagy function in the senescence inhibition event (Fig. 30.10).

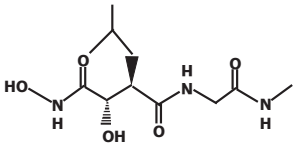


FIGURE 30.10 Chemical structure of marimastat.

2.22 Cyclo(RLsKDK)

Cyclo(RLsKDK) (BK-1361) as a specific ADAM8 inhibitor shows antiinflammatory diseases and cancer efficacy (Yim et al., 2016) (Fig. 30.11).

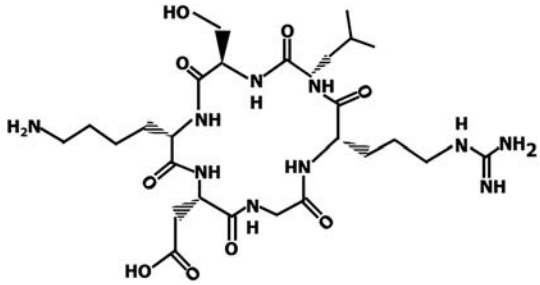


FIGURE 30.11 Chemical structure of cyclo(RLsKDK).

2.23 MMP-9-IN-6

MMP-9-IN-6 (3-indolyl furanoid) is an MMP-9 inhibitor that shows antiulcer effect (Rudra et al., 2023). 3-indolyl furanoid inhibits MMP-9 activity with a 50 μM IC_{50}, and it binds to the MMP-9 catalytic cleft (Fig. 30.12).

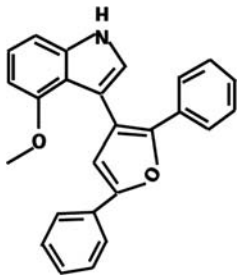


FIGURE 30.12 Chemical structure of MMP-9-IN-6.

2.24 N-isobutyl-N-[4-methoxyphenylsulfonyl]glycyl hydroxamic acid MMP-3 inhibitor

N-isobutyl-N-[4-methoxyphenylsulfonyl]glycyl hydroxamic acid (NNGH) is a prototype hydroxamate-based MMP-3 (stromelysin-1) inhibitor and inhibits Wnt/β-catenin signaling (Christianson et al., 2012). The peripheral hyperalgesia and tactile hypersensitivity were suppressed by intrathecal NNGH treatment (Fig. 30.13).

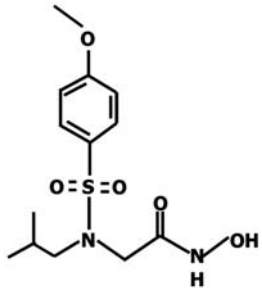


FIGURE 30.13 Chemical structure of NNGH.

2.25 Inotilone

Inotilone is an MMP-2/-9 inhibitor and inhibits metastasis of lung cancer cells. Inotilone activates the antioxidant enzymes to inhibit tumor metastasis and also inhibits IκBα phosphorylation and NF-κb p65 nuclear translocation (Chao et al., 2019) (Fig. 30.14).

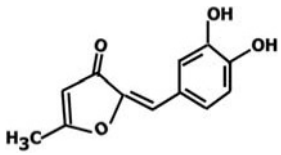


FIGURE 30.14 Chemical structure of Inotilone.

2.26 MMP13-IN-4

MMP13-IN-4, *N*-acyl hydrazine, is a selective and non—zinc-binding inhibitor of MMP-13 involved in OA (Cuffaro et al., 2023). MMP-13 is pivotal for OA and a therapeutic candidate. *N*-acyl hydrazine binds to the MMP-13 S1′ pocket. *N*-acyl hydrazone derivatives selectively inhibit the MMP-13 enzyme (Fig. 30.15).

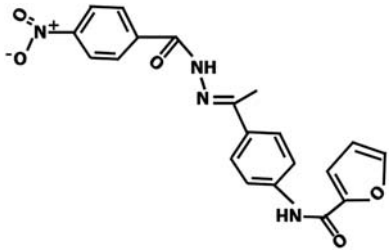


FIGURE 30.15 Chemical structure of MMP13-IN-4.

2.27 INCB3619

INCB3619 is an orally acting and selective ADAM10 and ADAM17 inhibitor. INCB3619 has antitumor activity in A549 xenografted BALB/c model mice (Zhou et al., 2006). INCB3619 suppresses heregulin-mediated HER3 signaling in A549 cells to induce apoptosis. It also diminishes epidermal growth factor receptor (EGFR) signaling in the EGFR-positive cells and combination therapy is potentiated with anti-EGFR tyrosine receptor kinase inhibitor (gefitinib) (Fig. 30.16).

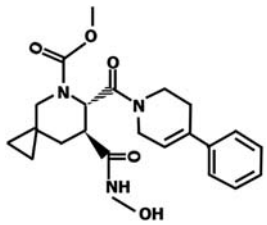


FIGURE 30.16 Chemical structure of INCB3619.

See Table 30.2.

3. Conclusion and emerged MMP-9 inhibitor

Recently, strategies have been changed to selectively inhibit certain type of MMP enzymes. The initial approaches of MMP inhibitors have mainly targeted the MMP active sites. For example, increased Zn-binding capacity of MMP inhibitors exhibited severe side effects and toxicity. Extracellular fluidal cytokines IL-1β and TNF-α are measured in LPS inflammatory model, and intracellular inflammasome formation is also detected. BBB permeability is detected by parallel artificial membrane permeability assay system. A specific MMP subtype has been targeted but not inhibition to all MMP types. Moreover, MMP active sites have not been targeted by MMP inhibitors, but the surrounded pocket and exocite have been targeted by inhibitors. In MMPs, prodomain's Cys residue binds to active site's zinc ion, which active site is not exposed. Several proteases such as furin, MMP-3, plasmin, and trypsin have been known to proteolytically remove the MMP protein prodomains, and the active site is exposed and proteinases show enzyme activities as in Fig. 30.17. Or, in vitro activators such as 4-aminophenylmercuric acetate or sodium dodecyl sulfate (SDS) and reactive oxygen products induce uncoupling of Cys-Zinc bonds to expose the active site and prodomain structures are cleaved to form matured active form.

For MMP-9 inhibitor screening in cell models, test-tube ELISA can be used for the pro-MMP-9 and inhibitor mixture, and then MMP-3 can activate MMP-9. The activated MMP-9 produces enzymatic digested products that are measured for MMP-9 inhibitor screening. In zymography, that MMP-9 substrate gelatin is added on SDS-polyacrylamide gel

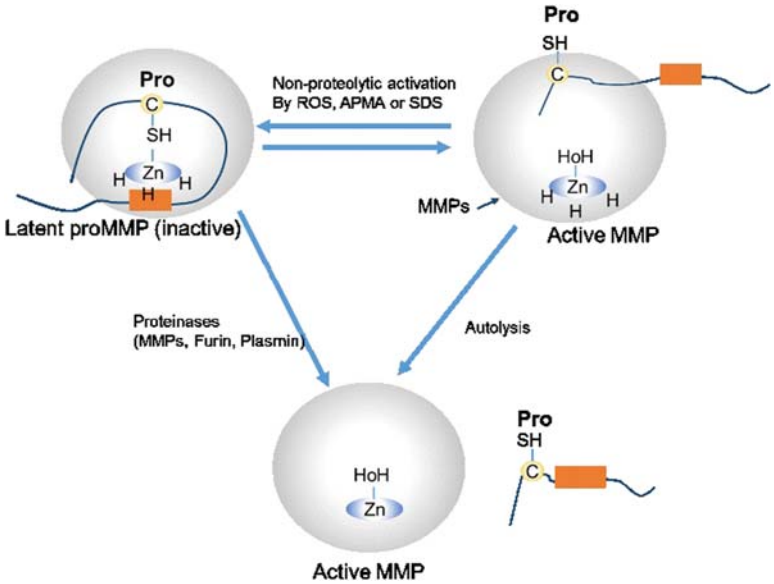


FIGURE 30.17 MMPs activation mechanisms. This figure shows how the inactive MMP is activated.

electrophoresis, pro-, intermediate- and mature-form of MMP-9 can be compared by MMP-9-drived digestion levels and inhibitor screening.

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CHAPTER

31

Evidence from clinical trials on synthetic protease inhibitors in oral, head, and neck cancer

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1. Introduction

Protease inhibitors are among the many natural and synthetic chemicals that have been investigated for use in various ranges of cancer treatments; they show promise as anticancer drugs. Using specialized inhibitors to impede the actions of dysregulated proteases can be a promising strategy for combating tumor growth and metastasis. While the advent of next-generation drugs eliminated the side effects of the early designs of these inhibitors, the majority of the suggested inhibitors failed early clinical trial stages because of nonspecific toxicity and a lack of pharmacological efficacy (Rudzińska et al., 2021).

Within the framework of cancerogenesis, the majority of extracellular proteases play a variety of roles in processes linked to the formation and advancement of tumors through distinct pathways. They can therefore serve as the foundation for the breakdown of membranes and matrices, the inactivation of natural protease inhibitors and chemotherapeutics, the control of cell viability, the modulation of the immune response, and the recruitment of inflammatory cells (Petushkova et al., 2019; Soond et al., 2019b). Furthermore, proteases found in the cytosol, nucleus, membrane, and mitochondria are linked to a variety of signaling pathways that they can stimulate, including adhesion, migration, proliferation, dedifferentiation, and the transition of cancer cells from epithelial to mesenchymal tissue. A variety of processes, including the activation of membrane receptors (such as the tumor necrosis factor receptor) or the production of reactive oxygen species, can lead to the release

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of proteases into different cellular compartments and their altered expression (Petushkova and Zamyatnin, 2020).

Collectively, the comprehensive exploration of the synthesis of peptidomimetic antagonists, regulating proteases, has yielded numerous promising avenues for cancer investigations (Mabonga and Kappo, 2020). However, the application of protease inhibitors in therapy is intricate due to the diverse utilization of carious proteases by different types of cancers at distinct stages of tumor progression. Consequently, a universal inhibitor applicable to all protease classes remains elusive. Successful development of suitable protease inhibitors necessitates the design and testing of small molecule inhibitors tailored to specific diseases and tissues (Kappelhoff et al., 2017; Fear et al., 2007).

In this context, transcriptomic and proteomic analyses play a pivotal role in validating inhibitors as specific targets. Furthermore, extensive experiments at molecular levels are imperative to elucidate the interplay between signal transduction pathways and protease activation cascades. This forms the foundation for maximizing inhibitor specificity and endorsing their repressive potential in vivo. Additionally, as a synthetic class of drugs, protease inhibitors must be meticulously designed with the appropriate conformation to establish prolonged interactions with specific proteases. Simultaneously, these designs must mitigate the risk of random degradation within the digestive tract, plasma, and intracellular environments (Dana and Pathak, 2020; Fear et al., 2007).

Moreover, challenges arise in the delivery of protease inhibitors, including issues related to low membrane permeability, rapid plasma elimination, high first-pass metabolism, very low oral bioavailability, rapid renal clearance, and short in vivo retention times. To overcome these hurdles, nanotechnology has been strategically employed to enhance the bioavailability of inhibitors at the target site, thus improving pharmacokinetic and drug performance properties. In the recent decade, innovative nanoparticle formulations, serving as nontraditional drug distribution platforms have gone through extensive investigation for their ability to selectively target and neutralize proteases (Dheer et al., 2019; Rizvi and Saleh, 2018).

Oral, head, and neck cancers pose a significant global health burden, necessitating innovative approaches for early detection and targeted therapeutic interventions. The 5-year survival rate for this type of cancer varies between 30% and 70%, depending on its stage and tumor location. It is crucial to address the fact that this type of cancer generally has a poor prognosis. The mean for incurable patients' survival is estimated to be 5 months with the duration ranging from weeks to more than 3 years. Part of the important factors impacting survival rate are the disease's stage at the time of diagnosis, the chosen treatment modalities, the primary tumor location, and individual-specific variables. Localized cancer survival rates are much higher, e.g., the survival rates for salivary gland and mucosal lip sites are over 90%, and laryngeal and hypopharyngeal sites are closer to 60% (Hoesseini et al., 2020). Research findings on synthetic proteases have opened new avenues for exploring novel diagnostic and therapeutic strategies in the context of these malignancies. This comprehensive review will further explore the emerging trends, methodologies, and potential applications of synthetic protease research in oral, head, and neck cancer.

2. Oral, head, and neck cancer

Oral, head, and neck cancers are the group of malignant tumors that involve the throat, larynx, nose, sinuses, and mouth. The majority of the cancers are squamous cell carcinoma (SCC) that originate from the flat squamous cells lining the mucosal surfaces in these areas. The predominant risk factors for these types of cancers include tobacco usage, alcohol abuse, and oncogenic viruses such as human papillomavirus (HPV) and Epstein−Barr virus (EBV). Generally, treating head and neck malignancies imposes significant challenges and hence requires multidisciplinary approaches that include surgery, radiotherapy, and systemic therapy (Mody et al., 2021).

The global incidence of HNC persists in its upward trajectory, with a projected annual increase of 30% expected by the year 2030. This escalation is evident for both developed and developing countries (Conway et al., 2018). Diverging from Southeast Asia, which faces the anticipated upsurge in oral cancer, the escalating incidence rates of HNC in the United States and Europe have been linked to a surge in oropharyngeal cancer, primarily associated to HPV infection. Recent findings revealed a global pattern characterized by a rising incidence in HPV-related subsites and a concurrent decline in HPV-unrelated subsites in nations such as the United States, Canada, Hong Kong, and Korea. Projections for the next two decades saddest that a predominant proportion of HNC cases will be HPV-positive. In certain European countries, such as the United Kingdom, it is estimated that the incidence of oropharyngeal cancer will surpass that of cancer in the oral cavity. Global incidence showed an increased incidence of laryngeal cancers by 23% over the past decade (Conway et al., 2018).

Let us delve further into the epidemiological landscape of oral cancers by looking into the global incidence, mortality rate, and demographic disparities. In 2020, the global burden of oral cancer surpassed 377,700 cases and ranked as the 16th most common cancer worldwide. Part of the countries that showed the highest rates of oral cancer are Papua New Guinea, Bangladesh, Romania, and Cuba. A staggering 573,775 men were diagnosed with the cancer in 2020, while approximately 171,219 women were diagnosed with the cancer, revealing notable gender variations. Furthermore, Bangladesh recorded the highest overall mortality rate of the specific cancer and closely followed by Papua New Guinea (Coppola et al., 2021). In developed countries such as the United States, previous projections indicated that an estimated 54,540 adults will be diagnosed with oral or oropharyngeal cancer in 2023, with men constituting the majority of the cases. Up to date, the actual number has not been published to validate the projections (Sankar and Xu, 2023).

The World Health Organization (WHO) advocates for the integration of oral health into universal health coverage by 2030, recognizing its pivotal role in overall health. An emphasis has been laid on early detection, awareness campaigns, and preventive measures to combat oral cancer and also other oral diseases on a global scale. The key measures are maintained for significantly reducing the risk of oral cancer, which include restraining from tobacco smoking and using areca nuts including betel quid with or without tobacco, and reducing alcohol consumption (Action Plan for Oral Health in South-East Asia 2022−2030: Toward Universal Health Coverage for Oral Health. World Health Organization, 2022).

2.1 Different regions affected by HNC

Regions categorized under HNC include the oral cavity, throat (pharynx), voice box (larynx), paranasal sinuses and nasal cavity, and salivary glands. It is crucial to emphasize that tumors affecting the brain, eye, esophagus, thyroid gland, and the skin of the head and neck are generally not categorized as HNCs. When SCC of the head and neck metastasizes, it typically does so within the local region or to the lymph nodes in the neck. Occasionally, cancerous squamous cells can be detected in the upper neck lymph nodes, even in the absence of apparent cancer elsewhere in the head and neck. The scenario is termed metastatic squamous cell carcinoma with an unknown (occult) primary (Arboleda et al., 2023).

2.2 Cancer

Mouth and oral cancers constitute a diverse array of malignancies affecting various sites within the oral cavity, such as the tongue, mouth lining, lips, gums, and the back of the throat. There are various types of oral cancers, with oral squamous cell carcinoma (OSCC) being the most common type of oral cancer (Bravi et al., 2021). The common issue shared by all types of oral cancer is that the first symptoms reported by patients are not specific for oral cancer but are also common in other oral diseases. Table 31.1 describes the different types of oral cancers based on cell types and location.

TABLE 31.1 Types of oral cancers based on cell types and location.

Oral cancer(cell types)	Characteristics
1. Squamous cell carcinoma (SCC)	• The most common type of oral cancer. • It develops within the thin squamous cells lining of the mouth and throat. Consequently able to spread into deeper tissues around the oral cavity. • This type of cancer is treatable at early stages with surgery and radiation therapy.
2. Verrucous carcinoma	• This is a rare subtype of SCC. • It is a slow-growing cancer that usually does not spread aggressively. • Due to its rarity, treatment recommendations found in literature are mostly based on case reports and small retrospective case series that are not uniform. • Treatment modalities include surgery, radiotherapy, chemotherapy, or combinations thereof.
3. Minor salivary gland carcinoma	• Relatively rare form of oral cancer. • Develops in the mouth of soft palate, sinuses, or voice box. • Treatment modalities include surgery, radiation therapy, and chemotherapy.
4. Lymphoma	• Affects the lymphatic system. • It is rare in the oral cavity but may occur in soft tissues of the mouth and jaw. • Treatment includes chemotherapy and radiation therapy.
5. Mucosal melanoma	• Consists of brown or black tumors that are relatively rare. • Suggestive treatment is surgical removal as chemotherapy shown to be less effective.

Adapted from references Markiewicz et al. (2022), Kristofelc et al. (2023).

The typical diagnostic method for oral cancer is made by tissue biopsy. However, recent advancements have led researchers to pay more attention to more rapid, economical, and noninvasive screening methods. A liquid biopsy, for example, showed promising clinical outcomes for early OSCC detection. It is a diagnostic method that detects biomarkers such as novel microbiome components, noncoding RNAs, extracellular vesicles, and circulating tumor DNA (Lousada-Fernandez et al., 2018). An optical detection system is noninvasive, convenient, and able to provide results in real time. Its capabilities to be repeated proved to be very useful in the diagnosis of oral cancer. The system uses light-based techniques for early cancer identification (Morikawa et al., 2020). Oral brush cytology collects cells from the oral cavity using a brush for analysis. The method was reported to have good accuracy for diagnosis of oropharyngeal and OSCC before treatment started, but its value declined once treatment started (Castillo et al., 2022). Microfluidic detection is a novel biological advancement based on the improvement of microsystems that can mimic and measure. It uses microfluidic devices to analyze biological samples collected (Akgönüllü et al., 2021). Recently, with the advancement of artificial intelligence (AI) auxiliary diagnosis, studies have demonstrated that AI approaches combined with imaging are able to have a considerable impact on oral cancer diagnosis and outcomes. Applications ranging from low-cost screening using smartphone-based probes to algorithm-guided detection have shown the ability to distinguish the heterogeneity and margins of the oral lesion using optical coherence tomography (Ilhan et al., 2020).

2.3 Standard therapies for HNC

Surgery is the primary treatment for localized HNC, with the aim of removing the tumor and achieving clear margins. The success rate is high, and a complete cure can be reached in the early stages of the disease. However, postsurgical wound healing issues, nerve damage, and functional deficits are some of the undesirable complications postsurgically. Radiation therapy or radiotherapy targets cancer cells with high precision (Mody et al., 2021). Its usage can be bidirectional, either as the primary treatment of adjuvant therapy after surgery. The treatment is found to be effective for both localized and locally advanced HNC. There are side effects of mucositis, xerostomia (dry mouth), skin changes, and long-term complications of the radiotherapy. Despite its benefits, radiotherapy may affect adjacent healthy tissues (Mody et al., 2021) (Rosenberg and Vokes, 2021).

Another treatment option that is used in combination with surgery or radiotherapy is chemotherapy. The treatment proves to be able to shrink tumors before surgery (neoadjuvant) or prevent recurrence (adjuvant). It is a systemic treatment for metastatic or recurrent HNC. Patients who undergo the therapy commonly complain about its toxicity, and effects, which lead to nausea, extreme fatigue, hair loss, and myelosuppression. To date, chemotherapy has shown limited efficacy as monotherapy (Mody et al., 2021; Mesia et al., 2021).

Targeted therapies emerged as a promising modality in the late 20th century as scientists identified specific molecules and pathways integral to cancer growth and progression. Research efforts have concentrated on the development of drugs selectively inhibiting these identified targets. Clinical trials have played a pivotal role in evaluating the efficacy and safety of targeted therapies. These trials, involving head and neck cancer patients, have systematically assessed the impact of targeted drugs on parameters such as tumor growth,

survival rates, and quality of life, which has led to US Food and Drug Administration (FDA) approvals on several targeted drugs. Targeted therapy exemplifies the concept of personalized medicine. Physicians analyze the genetic and molecular characteristics of a patient's tumor to determine the most suitable targeted drug tailored to the individual's unique profile. Currently, targeted therapies stand as a standard treatment option for specific head and neck cancer cases. The choice of therapy is contingent upon factors such as tumor type, stage, and individual patient characteristics. Some of the approved targeted therapies for HNC are cetuximab (Erbitux) that targets the epidermal growth factor receptor, and immunotherapies, which include nivolumab (Opdivo) and pembrolizumab (Keytruda). Both nivolumab and pembrolizumab are immune checkpoint inhibitors that enhance the body's immune response against cancer cells (Nakano, 2021; Taberna et al., 2019).

Immunotherapy has harnessed recent interest among researchers in HNC treatment. Immunotherapy is different from traditional chemotherapy because it specifically focuses on the immune system rather than directly killing cancer cells (Pei et al., 2021). Synthetic protease inhibitors, traditionally associated with cancer treatment, have recently gained attention due to their immunomodulatory effects; hence, they are categorized as part of immunotherapy (Nam et al., 2020).

3. Understanding proteases and their role in head and neck cancers

As previously discussed, HNC is a complex disease that arises from a multistage development process. Survival of the cancerous cells depends on the tumor ecosystem, which promotes their growth and shields them from immune surveillance. According to EUROCARE, most lethal HNC tumors are located in the hypopharynx (25%), larynx (59%), oropharynx (39%), tongue (43%), oral cavity (45%), and nasopharynx (49%) (Gatta et al., 2015).

Tumor dissemination and metastasis constitute hallmark processes associated with cancer relapse and treatment failure, contributing to approximately 90% of cancer-related deaths. Metastasis, the final outcome of a complex cellular progression known as the metastatic cascade, involves the dissemination of tumor cells to distant organs and their adaptation to diverse microenvironments. Each stage in this cascade is guided by the acquisition of genetic and/or epigenetic alterations, distinctive to tumor cells or stromal cells, bestowing upon the invading cells the attributes necessary for the development of macroscopic metastases. Recent advancements have elucidated the molecular changes associated with these events, offering insights into the key determinants influencing metastatic progression, thereby presenting potential therapeutic targets. Ongoing research addresses the idea that synthetic protease inhibitors are a potential avenue for targeted treatment in HNC by modulating critical cellular pathways (Peltanova et al., 2019). Some of the key proteases associated with HNC cancer include matrix metalloproteinases (MMPs), cathepsins, serine proteases, kallikreins, and cysteine proteases. Let us delve into the intricate connections between proteases and HNC.

3.1 Matrix metalloproteinases and tissue inhibitors of metalloproteinases

The MMPs are a family of proteolytic enzymes that were found to exhibit aberrant gene expression, in various cancers. They are involved at many levels of carcinogenesis, due to

their role in cell growth, differentiation, apoptosis, migration, invasion, angiogenesis, and immune surveillance. While numerous investigations have explored MMPs in the context of head and neck squamous cell carcinoma (HNSCC), these studies have predominantly concentrated on MMPs associated with invasion and metastasis. Despite such efforts, the outcomes often exhibit a proclivity for inconsistency (Maurya et al., 2017).

MMPs play a crucial role in degrading extracellular matrix proteins (ECM), and basement membrane, and important molecules for growth factors, cell surface receptors, and adhesion molecules. Originally, the primary hypothesis regarding MMP function focused on their capacity to degrade ECM, facilitating tumor cell migration to local and distant sites. Recent observations highlight the critical role of MMPs in the ECM, where they activate or release growth factors, thereby promoting the initiation and proliferation of primary tumors. MMPs are also implicated in tumor angiogenesis by activating basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), and transforming growth factor-beta (TGF-β). Previously, the tumor cells were regarded as the primary source of MMPs in the stroma for ECM degradation; however, recent findings showed that they were predominantly produced by the stromal cells within the tumor microenvironment, such as fibroblasts and inflammatory cells (Maurya et al., 2017).

The regulation of MMPs is governed by tissue inhibitors of metalloproteinases (TIMPs), influencing the breakdown of the extracellular matrix (ECM). This equilibrium between MMPs and TIMPs plays a pivotal role in maintaining the integrity of tissues and is crucial in various pathological conditions. There are four distinct TIMPs that have been identified: TIMP-1 (28.5 kDa), TIMP-2 (22 kDa), TIMP-3 (22 kDa), and TIMP-4 (22 kDa) that are produced by various cell types (Hua et al., 2011).

In HNC, studies have extensively examined the involvement of MMPs in invasion and metastasis. An essential event in the initiation of invasion is the signaling of the metastatic cascade through the interaction of tumor cells with the basement membrane (BM). Tumor cells instigate ECM degradation by engaging in BM proteolysis, facilitating tumor cell propagation. This ECM degradation also induces a significant phenotypic alteration in the cells, characterized by the acquisition of a mesenchymal profile, a process primarily governed by MMPs and commonly known as the epithelial—mesenchymal transition (EMT) (Mughees et al., 2021).

About a decade ago, in 2006, Görögh et al. concluded that MMP-2 overexpression exhibited a correlation with metastasis, while elevated levels of TIMP-1 and -2 were associated with a decrease in tumor growth (Görögh et al., 2006). The conclusion is still considered as an important basis in current ongoing studies. Higher levels of MMP-2 and MMP-9 were identified at metastatic sites than in the primary tumor, with statistically significant differences found in both T1 and T2 cases. Contrary to the high expression of MMPs in metastatic regions, tumor-associated macrophages were predominant in the primary regions, implying an inverse relationship between tumor-associated macrophage numbers and MMP expression levels in primary and metastatic regions (Nishio et al., 2016). A recent study by Ren et al. (2020) reported that the mRNAs of MMP-7, MMP-13, and MMP-10 were subject to regulation, with MMP-12 and MMP-9 remaining unregulated in metastatic tumors compared with their nonmetastatic counterparts. This suggests the presence of genes that play crucial roles in metastasis through the regulation of MMP-7 and MMP-13 (Ren et al., 2020).

A recent study in 2023 observed that within 56.2% of cases, MMP-9 expression correlated more strongly with the presence of lymph node metastases, advanced cancer stage, and tumor grade (Chakraborty et al., 2023). The study corresponds to previous findings that concluded MMP-2 and MMP-9 were overexpressed in patients with lymph node involvement, and MMP-9 was associated with a low survival rate, indicating a poor prognosis. Targeting the remodeling of the HNC cell carcinoma microenvironment has been proposed as a therapeutic strategy to prevent lymph node metastasis. MMP-9 and MMP-14 were found to be upregulated in lymph nodes involved in metastasis and closely positively correlated with the level of ALG-2 interacting protein X (ALIX), a protein promoting the migration and invasion of HNSCC cells. ALIX expression in HNSCC was statistically higher than in normal mucosa. MMP-9 and MMP-14 localized in the cytoplasm of cancer cells significantly enhanced in metastatic lymph nodes compared with the primary tumor. The degradation of the extracellular matrix caused by MMPs is deemed critical, and the results suggest that ALIX may contribute to increased MMP-9 and MMP-14 levels, thereby facilitating lymph node metastasis. Data from The Cancer Genome Atlas (TCGA) further validated the correlation between ALIX and MMP-14, although no correlation was identified with MMP-9 (De Vicente et al., 2008; Xie et al., 2023).

Another concern highlighted by various studies is that radiation is able to upregulate various MMPs, despite the fact that radiotherapy is a prevalent treatment modality for HNC. It was suggested that there is an increased risk of metastasis in cells surviving radiotherapy due to the activation of MMPs (Brandt et al., 2023).

Beyond any doubt, the MMP family plays a multifaceted role in HNC, influencing tumor proliferation, EMT, angiogenesis, invasion, and metastasis. Existing findings indicate that patients with HNC may exhibit distinct MMP profiles based on the metastatic stage of the cancer, the primary tumor site, the tissue type of origin, or genomic disparities among patients. The expression of MMPs has been linked to a poorer prognosis for HNC patients. While the precise mechanistic significance of the MMP family as a therapeutic target and prognostic biomarker in HNC is not yet fully elucidated, current findings suggest that certain MMPs have diagnostic, prognostic, and therapeutic potential for the HNC patients (Fornieles et al., 2024).

3.1.1 Human studies on MMP inhibitors in cancer therapy and HNC

Between the 1990s and the early 2000s, synthetic inhibitors of MMPs, known as MMPIs, were extensively investigated across various cancer types. Despite promising preclinical data, all trials during this period proved unsuccessful in reducing tumor burden or improving overall survival. Furthermore, the use of MMPIs led to unexpected and severe side effects. Two primary factors can account for the failure of MMPIs in clinical trials. Firstly, it has become evident that certain MMPs possess antitumor effects. Therefore, the broad-spectrum MMPIs utilized in the initial trials may have inadvertently blocked these beneficial MMPs, potentially contributing to tumor progression (Winer et al., 2018).

Several MMPIs were synthesized, and clinical trials have been conducted since the 1980s; however, there is no reported recent data on trials involving HNC. Table 31.2 illustrates the early reported outcomes of MMPIs clinical trials. One of the earliest drugs developed was batimastat. Despite the preclinical study showing potential of antitumor effect, its toxicity resulted in a halt to further testing. Failure of next-generation broad-range inhibitors led to

more selective inhibitors (Parsons et al., 1997; Lombard et al., 1998; Macaulay et al., 1999; Bramhall et al., 2002; King et al., 2003; Miller et al., 2004; Sparano et al., 2004; Rosenbaum et al., 2005; Heath et al., 2006; Hirte et al., 2006; Levin et al., 2006; Rasmussen and McCann, 1997; Leighl et al., 2005; Bissett et al., 2005; Lara et al., 2006).

Other ongoing trials involving the MMPIs AB0041, AB0046, and GS-5745 reported early results, hoping to see the rebirth of MMPIs in cancer therapy. The AB0041 preclinical studies demonstrated its efficacy in inhibiting tumor growth and metastasis in a mouse xenograft model of colorectal carcinoma; thus, it is a long way to go to evaluate its clinical potential (Fields, 2019). AB0046 also focuses on a monoclonal antibody designed to inhibit MMP-9. Similarly, preclinical studies using knockout mice and a mouse MMP-9 protein that composed of the pro- and catalytic domains only showed promising results and have yet to go through extensive research to get to clinical trials (Marshall et al., 2015). The GS-5745 utilizes a model of colorectal carcinoma that has demonstrated efficacy in inhibiting tumor growth and metastasis. Additionally, it proves to be able to selectively inhibit MMP-9 in colorectal tumor burden in murine orthotopic tumor models. This is the first anti-MMP antibody that is currently undergoing clinical investigation and is known as andecaliximab (clinical trial number: AB0041) (Waller and Pruschy, 2021) (Table 31.2).

3.1.2 Conclusions on MMPIs potential in cancer and HNC therapy

Since the failure of multiple trials in this arena, compounds have been shelved and trials have been put on hold. Furthermore, there is no reported data on specific findings of this type of protease inhibitor in HNC patients. Prior clinical trials involving other types of cancer patients provide valuable lessons that include the need for selective and metabolically stable inhibitors. Despite the widespread use of mouse models to study in vivo MMP behaviors, it was found that these models may not reliably reflect human conditions and disease mechanisms. Recent genetics and proteomics approaches provide a clearer understanding of the impact of MMP activity on human pathophysiological systems. The consideration of MMP secondary binding sites (exosites) represents a promising avenue for the development of selective inhibitors. The FDA approval of an MMP inhibitor and the progression of numerous MMP inhibitors to clinical trials indicate that it is time to deviate from the traditional perspective that MMP inhibition is an intractable challenge. The evolution in the understanding of MMPs and the advancements in inhibitor design suggest a renewed optimism for exploring MMP inhibition as a viable therapeutic strategy.

3.2 Cathepsins

Cathepsins are part of lysosomal proteases family that primarily reside within lysosomes, a specialized cellular compartment responsible for protein degradation. Typically, cathepsins are active in acidic environments. There are generally a dozen members in cathepsin family with each of it has distinct properties including serine proteases (cathepsin A, G), cysteine proteases (cathepsin B, C, F, H, K, L1, O, S, W, Z), and aspartyl proteases (cathepsin D, E). Table 31.3 describes the cathepsin family that implicates in context of cancer.

Cathepsin B (CatB) has garnered significant attention due to its upregulation across various cancers, demonstrating a close association with cancer development and metastasis. Consequently, CatB is increasingly recognized as a promising target for tumor imaging and

TABLE 31.2 Early reports on MMPIs cancer clinical trials.

Name of MMPI (references)	Type of inhibitor	Target MMPs	Type of cancer trials	Toxicity	Outcome
Batimastat Parsons et al. (1997), Macaulay et al. (1999), Rasmussen and McCann (1997)	Hydroxymate (zinc chelator)	Broad range (MMP-1, -2, -3, -7, -9, -14)	Malignant ascites (pancreatic, colorectal, gastric, ovarian, cholangiocarcinoma, ovarian, mesothelioma), malignant pleural effusion (non–small-cell lung, breast, melanoma, renal, mesothelioma)	Musculoskeletal syndrome, fever, liver function abnormalities, pleural pain at site of injection, GI upset	Canceled in phase III clinical trials (marimastat developed)
Marimastat Bramhall et al. (2002), Sparano et al. (2004), King et al. (2003), Rosenbaum et al. (2005); Levin et al. (2006)	Hydroxymate (zinc chelator)	Broad range (MMP-1, -2, -3, -7, -9)	Breast, non–small-cell lung, colorectal, pancreatic, gastric, prostate, glioblastoma	Musculoskeletal syndrome, GI upset	Canceled in phase III clinical trials
Tanomastat Lombard et al. (1998), Hirte et al. (2006)	Carboxylate (zinc chelator)	Broad range (MMP-2, -3, -8, -9, -13)	Pancreatic, ovarian, small-cell lung	Hematologic (anemia, thrombocytopenia), electrolyte abnormalities, hyperbilirubinemia, GI upset	Canceled in phase III clinical trials
Prinomastat Lombard et al. (1998), Bissett et al. (2005), Heath et al. (2006)	Hydroxymate (zinc chelator)	Broad range (MMP-2, -3, -9, -13, -14)	Non–small-cell lung, esophageal	Musculoskeletal, venous thromboembolism, hematologic, GI upset	Canceled in phase III clinical trials
Rebimastat Lombard et al. (1998), Leighl et al. (2005), Miller et al. (2004), Lara et al. (2006)	Sulfhydryl-based mercaptoacyl zinc chelator	Broad range (MMP-1, -2, -3, -8, -9, -13, -14)	Non–small-cell lung, prostate	Dermatologic, hypersensitivity	Canceled in phase III clinical trials
Andecaliximab *Andecaliximab Overview* (2024)	Monoclonal antibody	MMP-9	Gastric, breast, pancreatic, non–small-cell lung, esophageal, colorectal	Neutropenia, nausea, pain, GI upset	Completed phase III (suggested to evaluate the efficacy and safety specifically in advanced gastric or gastroesophageal junction adenocarcinoma)

TABLE 31.3 Cathepsin family that implicates in context of cancer.

Cathepsin	Functions and its implication in cancer	References
B	Beta-secretase, cleaving amyloid precursor protein to produce amyloid beta. Upregulated across various types of cancer. Closely related to cancer development and metastasis.	Shen and Li (2022)
H	Lysosomal protein degradation. Plays a role in tumor progression and invasion.	Linders et al. (2023)
K	It is essential for bone resorption. Implicates in cancer associated to bone metastasis.	Linders et al. (2023)
L	L1 implicates in matrix degradation and cell invasion. L2 involves in lysosomal protein degradation. It drives tumor progression, proliferation, and invasion.	Linders et al. (2023)
S	Relevant in immune responses. Involves in matrix degradation and cell invasion. It represents a promising target for cancer immunotherapy.	Fuchs et al. (2020)

cancer treatment. Imaging tools designed to target CatB can facilitate the identification of tumor boundaries, thus aiding surgical navigation (Linders et al., 2023).

There are prodrugs, and other therapeutic agents designed to respond to CatB have been proposed and utilized in clinical studies, with some already available on the market. This underscores the potential of targeting CatB as a valuable strategy in both diagnostic and therapeutic aspects of cancer management (Shen and Li, 2022).

Cathepsin H (CatH) and cathepsin L (CatL) are part of cysteine cathepsins that are expressed ubiquitously and share a broad spectrum of substrates. On the contrary, cathepsin K (CatK) and cathepsin S (CatS) are substrate specific and only being expressed by certain cell types (Linders et al., 2023). During tumor progression, the activity of cysteine cathepsins, which are typically and tightly regulated, undergoes alterations due to gene amplifications and the formation of transcript variants. It was also previously highlighted that there is a shift in the ratio between cysteine cathepsins and their endogenous inhibitors, such as cystatins and stefins, leading to an upregulation of cathepsin activity. The abnormal cathepsin activity is not confined to a particular cancer type and occurs in both tumor cells and tumor-associated cells, including fibroblasts, myoepithelial cells, endothelial cells, and various immune cells, with a particular emphasis on tumor-associated macrophages (TAMs). The expression pattern of these cysteine cathepsins in tumor tissue and the degree of their upregulation vary across different types of cancer. Hence, current information underscores the need for future research on cysteine cathepsins that are cancer-type-specific (Linders et al., 2023) (Soond et al., 2019a,b).

CatS is also part of the cysteine protease known for cleaving specific extracellular matrix proteins, regulating antigen presentation in antigen-presenting cells (APCs), and fostering the polarization of M2-type macrophages and dendritic cells. Overexpression of CatS is observed in many solid cancers, and it generally contributes to the establishment of an immune-suppressive and tumor-promoting microenvironment. While existing data suggest that inhibiting or knocking down CatS promotes anticancer immunity, therapeutic efficacy seems to hinge on cell-specific inhibition, particularly in myeloid cells (Fuchs et al., 2020).

Designing CatS-selective inhibitors and directing them toward tumor-associated M2-type macrophages (TAM) and dendritic cells emerges as an appealing therapeutic strategy, offering advantages over nonselective immunosuppressive compounds or untargeted approaches. The first compilation of selective CatS inhibitors was reviewed in 2004. Despite similarities with other cathepsins (K, L, B), CatS has distinctive features in its S2 and S3 pockets, offering potential for selective inhibition. The unique characteristics of CatS's S2 pocket, especially the flexible Phe211, allow for the exploration of larger substituents, making the search for selective inhibitors more attractive compared with other cathepsins such as CatK. Achieving selective CatS inhibition can be realized through optimized small molecule inhibitors exhibiting favorable pharmacokinetic profiles and oral bioavailability. Moreover, targeting these inhibitors to TAM is becoming more feasible using nanocarriers designed for directed delivery. The role of CatS in the immunological tumor microenvironment explores emerging possibilities for nanocarrier-mediated delivery of potent and selective CatS inhibitors to TAM and related APCs to enhance antitumor immunity (Shen and Li, 2022; Linders et al., 2023; Fuchs et al., 2020; Korkmaz et al., 2021; Soond et al., 2019a,b).

3.2.1 Cathepsin and HNC

CatL has been reported to be part of protein in the intricate network of molecular events underlying HNC progression. It plays a crucial role in the regulation of cancer progression through its involvement in extracellular matrix degradation, cell invasion, and metastasis. Besides, CatL, CatD, CatK, and CatS were also found to be linked to HNC progression. In prior studies, CatD received significant attention in breast cancer research. Nevertheless, it has been discovered that a heightened concentration of CatD is present in five lymph node metastases of HNC, underscoring its significance in the context of HNC. The role of CatK in OSCC takes center stage as it emerges as a significant factor associated with lymph node metastasis and prognosis in this form of cancer. The ability of cysteine cathepsins, particularly cathepsin K, to actively participate in the breakdown of the extracellular matrix underscores their involvement in tumor invasion and metastasis. The correlation between elevated levels of CatK, lymph node metastasis, and prognosis in OSCC suggests its potential as a prognostic marker and therapeutic target.

Another family member of cysteine cathepsin, namely the CatS, involves in the intricate interplay with interleukin-7 (IL-7); hence, it adds another layer of complexity to the molecular landscape of HNC. Notably, in HNC cell lines, the expression of CatS has been observed to lead to a significant reduction in the secretion levels of IL-7. The mechanism involves CatS-mediated modulation of the IL-7 receptor, disrupting its normal function and subsequently influencing the activation of lysosomal degradation pathways. Besides that, generally, CatS was also reported to be involved in degrading the extracellular matrix, promoting angiogenesis, and fostering metastasis. Ongoing research proposes CatS as a potential therapeutic target for cancer treatment (Zeillinger et al., 1992; Leusink et al., 2018; Chang et al., 2023; Gao et al., 2023).

3.2.2 Conclusions on cathepsin inhibitors in cancer and HNC therapy

Cathepsin inhibitors that have undergone clinical trials in cancer research include CatB inhibitors with potential for cancer therapy and imaging in melanoma patients (Matarrese et al., 2010) and CatX inhibitors as selective inhibitors in breast cancer patients (Mitrović et al.,

2022). CatS, on the other hand, is currently receiving attention as it has novel potential to be part of immunotherapy through nanocarrier-mediated delivery (Fuchs et al., 2020).

In summary, while cathepsins play a crucial role in the progression of head and neck cancer (HNC), influence immune responses, and may impact susceptibility to infections, the therapeutic use of cathepsin inhibitors for HNC remains a distant prospect. This is primarily due to the limited availability of clinical trials and experimental studies specifically involving HNC. Additional research is imperative to elucidate the exact roles of cathepsins and their therapeutic implications in the context of head and neck cancer.

3.3 Serine proteases

Serine proteases constitute the largest family of human proteases, with 175 predicted members. They were characterized by the presence of a nucleophilic serine (Ser) within their substrate-binding sites. The presence of Ser in their active site enables the cleavage of peptide bonds at the carboxyl terminus, resulting in the formation of an acyl–enzyme intermediate. The activity of serine proteases is regulated at multiple levels, including transcriptional, posttranscriptional, and posttranslational mechanisms. Typically, these enzymes function as zymogens to control their enzymatic activities (Kahler et al., 2020).

Serine proteases are involved in numerous essential physiological and developmental processes in humans, including cellular growth, differentiation, and development, as well as protein digestion, blood coagulation, and regulation of the complement system. Serine proteases in the human body fall into two main categories based on their structure; chymotrypsin-like/trypsin-like (cleave peptide bonds following positively charged amino acids such as lysine or arginine) and subtilisin-like (Kahler et al., 2020).

A subgroup of these proteases is directly anchored to plasma membranes, known as cell surface–anchored serine proteases, and it plays pivotal roles in various facets of cancer development and progression. Structurally, these enzymes are characterized by two beta-barrel domains converging at the catalytic active site. They are classified into type I, tethered to the plasma membrane via a carboxy-terminal transmembrane domain; type II, anchored by an amino-terminal proximal signal anchor that functions as a transmembrane domain; and GPI-anchored, attached via a carboxy-terminal hydrophobic region and a glycosylphosphatidylinositol linkage. Functionally, cell surface–anchored serine proteases are implicated in cancer-related processes such as tumor initiation, cancer cell migration and invasion, tumor vascularization (angiogenesis), and modulation of immune responses within the tumor microenvironment (Rachel and Sirisha, 2017).

Notable examples include matriptase, overexpressed in breast and prostate cancers; hepsin, associated with hepatocellular carcinoma and prostate cancer; and TMPRSS4, linked to pancreatic cancer progression. Understanding the diverse biological roles and structural characteristics of these proteases provides insights into potential targets for therapeutic interventions in cancer (Rachel and Sirisha, 2017).

Kallikreins are a family of trypsin-like secreted serine proteinases. These enzyme dysregulations were also associated with cancer progression. Tissue kallikreins (hKs) represent a significant subgroup within the S1 family of serine proteases, encoded by 15 structurally similar genes (KLK) located on chromosome 19q13. This genomic arrangement forms the largest contiguous cluster of protease genes in the human genome. With widespread expression

across various tissues, hKs participate in diverse physiological functions, including blood pressure regulation, electrolyte balance, tissue remodeling, prohormone processing, neural plasticity, and skin desquamation. Certain kallikreins, such as hK3/PSA (prostate-specific antigen), have emerged as promising diagnostic and prognostic biomarkers for ovarian, breast, and prostate cancers. Moreover, evidence suggests that hKs may actively contribute to carcinogenesis, especially in tumor metastasis and invasion (Martin and List, 2019).

3.3.1 Studies on serine proteases inhibitors in cancer therapy and HNC

In recent years, various research projects that have focused on developing strategies to target serine proteases for therapeutic purposes. Serpins represent serine protease inhibitors that possess the ability to covalently bind to serine proteases, effectively arresting their activity. There is a vast range of cancer research targeting serpins as the potential therapeutic applications, and certain serpins have been linked to the progression or remission of specific cancers. Table 31.4 lists different types of serpins and their associated cancer therapeutic research (Martin and List, 2019; Zheng et al., 2013).

In HNC, utilization of serine inhibitors as the therapeutic options is heavily studied at the preclinical stage. For example, headpin, a recently identified serpin protein, has emerged as a key player in regulating protease activity within the tumor microenvironment. This novel serpin is expressed in normal oral epithelium, where it acts as an inhibitor of protease enzymes. Intriguingly, its expression is notably absent in invasive head and neck squamous cell carcinoma (HNSCC). Given the critical role of proteases in facilitating invasion and metastasis through extracellular matrix lysis, headpin's absence in HNSCC suggests potential implications for cancer progression. Given its role as a protease inhibitor, headpin represents an important therapeutic target for treatment of cancers. Strategies aimed at restoring or mimicking headpin expression in cancerous tissues may offer a novel approach to mitigating the aggressive behavior of tumors. Small molecules, gene therapies, or immunotherapeutic approaches targeting headpin expression or enhancing its inhibitory activity could be explored for their efficacy in controlling cancer progression (Mazumdar et al., 2005) (Fig. 31.1).

TABLE 31.4 Different types of Serpins and their associated cancer therapeutic research.

Serpin type	Associated cancer therapeutic research	Level of study
PEDF (pigment epithelium-derived factor)	Antiangiogenesis activity: PEDF has potent antiangiogenesis properties, which can help inhibit blood vessel formation in tumors. It also promotes cancer cell apoptosis.	In vitro, In vivo
Neuroserpin	Tumor-suppressive functions. Potential target for antiproliferative therapies.	In vitro, In vivo
Serp-1 (derived from myxoma virus)	Tumor-suppressive functions.	In vitro, In vivo
Plasminogen activator inhibitor-1 (PAI-1 or SERPINE1)	Altered levels of PAI-1 are detected in cancer diseases. It may drive excess arterial and vascular plaque growth, thrombosis, and cancer invasion and metastasis.	In-vivo

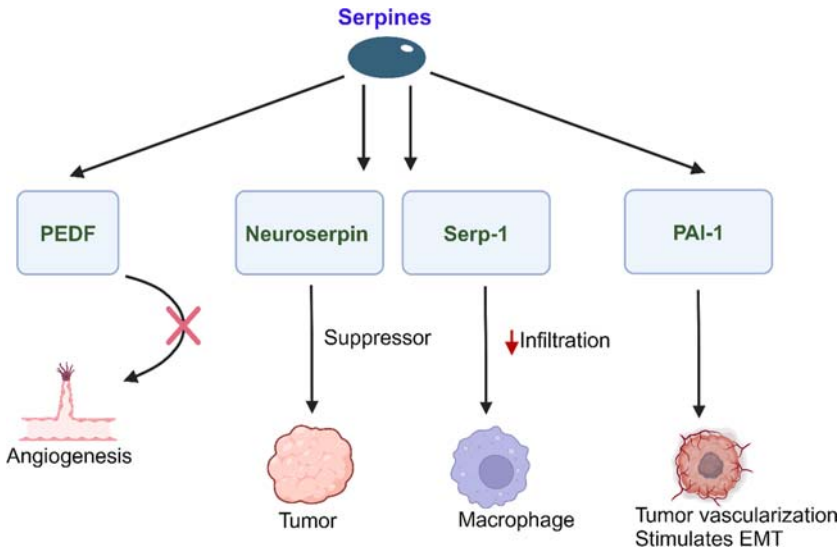

FIGURE 31.1 Schematic diagram showing actions of different types of serpins. Created with BioRender. The different types of serpins have been used in treatment of head and neck cancers. The schematic diagram shows different serpins.

Names of different serpins can be seen.

3.3.2 *Conclusions on serine protease inhibitors in cancer and HNC therapy*

Researchers are actively developing serine protease inhibitors to modulate their activities in tumor progression. These inhibitors show promising potential in blocking tumor growth by preventing protease-mediated extracellular matrix degradation, reducing metastasis by inhibiting invasion-associated proteases, and enhancing treatment response by sensitizing cancer cells to chemotherapy or radiation.

Headpin, a serpin protein, emerges as a promising protease inhibitor in HNC. Despite the progress, challenges persist in identifying specific protease targets and developing selective inhibitors. Combination therapies involving serine protease inhibitors could enhance treatment efficacy. Continued research is crucial for translating these findings into effective clinical interventions. In conclusion, serine protease inhibitors stand as potential adjuncts to conventional cancer therapies. Their precise targeting, along with validation in clinical trials, holds the key to shaping the future of cancer treatment.

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CHAPTER

32

Synthetic protease inhibitors in endocrine and gastroenterology cancers: Results of clinical trials

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1. Introduction

The cells of the endocrine organs secrete different hormones. In the cells of an endocrine organ, there may be abnormal growth, which is seen in a tumor. Tumor in endocrine organs comprises both benign and malignant types. A functional tumor may produce hormones while a nonfunctioning may not produce hormones. The neuroendocrine tumors affect the nervous system of the body (the neuroendocrine tumors secrete specific hormones that produce varied systemic manifestations). The neuroendocrine tumors affect the nervous system of the body. The endocrine system comprises the thyroid, pituitary, thymus, adrenal, pancreas, and ovary. The major gastrointestinal cancers include malignancy of esophagus, stomach, liver, colon, and pancreas. In this chapter, we discuss the pathophysiology of endocrine and gastroenterology cancers and discuss the potential role of protease inhibitors as promising therapeutic agents.

1.1 Endocrine cancers

1.1.1 Thyroid cancer

According to the GLOBOCAN 2020 database of cancer incidence and mortality by the WHO International Agency for Research on Cancer, thyroid cancer was the ninth highest cancer in the world (Sung et al., 2021). Thyroid cancer was reported to be the fifth most common cancer in females in the USA, and an estimated over 62 ,000 new cases were reported in males and females in 2015 (Cabanillas et al., 2016).

The majority of the thyroid cancers are well-differentiated thyroid cancers, which accounts for more than 95% of cases (Howlader et al., 1975), and it originates from follicular epithelial

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cells of the thyroid. Under the category of well-differentiated thyroid cancers, papillary thyroid cancer, follicular thyroid cancer, and Hurthle cell thyroid cancer are included. Other types include medullary thyroid carcinomas and anaplastic thyroid carcinomas. Hurthle type and anaplastic types are less common. Papillary type of thyroid carcinomas have good prognosis. Physical examination, measurement of level of thyroid stimulating hormone (TSH), biopsy, ultrasound, and radioactive scans aid in the diagnosis. Management involves thyroidectomy, lobectomy, involved lymph node dissection, and hormone therapy. For anaplastic types, latest therapy such as immunotherapy may work well. Protease inhibitors may also be beneficial intreating thyroid cancers.

1.1.2 Pituitary cancer

Among the intracranial tumors, pituitary gland tumors are quite common. The tumors that arise from the anterior lobe are benign, and when the tumors metastasize, they are known as pituitary carcinoma (Butt et al., 2023). At times, there is also metastasis to the pituitary gland. The exact etiology of the tumors of the pituitary gland is unknown, but it may be genetic. Mutations in the genes and formation of oncogenes may be important events. Pituitary tumors, which were associated with specific germline mutations such as MEN1, AIP, and GPR101, were found to be more aggressive and resistant (Srirangam Nadhamuni and Korbonits, 2020). Fortunately, the prevalence of pituitary cancer is very low. The tumor is usually seen after the age of 40 years. Common investigations include hormone estimations, neuroimaging, and neuroophthalmic examination. Common investigations include hormone estimations, neuroimaging, and neuroophthalmic examination. Management includes surgery, radiotherapy, chemotherapy, and latest immunotherapy.

1.1.3 Adrenal cancer

Adrenal carcinomas are rare but may be aggressive in nature. Other types include neuroblastoma and pheochromocytoma. According to reports, the worldwide incidence of adrenal carcinomas is 0.5−2 per 1 million individuals annually and accounts for 0.02%−0.2% of all cancer-related deaths (Torti and Correa, 2023). The exact cause of adrenal carcinomas is unknown. Etiology includes gene mutation. Hormonal investigations, blood, urine investigations, and radiological imaging may aid in diagnosis. Management involves surgery, radiotherapy, chemotherapy, and immunotherapy. In immunotherapy, the immune checkpoints inhibitors are useful. In immunotherapy, the immune checkpoints are blocked. The progression of tumor and resistance may be a challenge in the treatment of adrenal carcinomas.

1.1.4 Pancreas cancer

The pancreas is an organ, which lies retroperitoneal and has both exocrine and endocrine functions. The tumors of the pancreas are mainly divided into nonendocrine and endocrine types. Pancreatic cancer includes ductal adenocarcinoma and cystadenocarcinoma. Pancreatic ductal adenocarcinoma is the commonest pancreatic cancer. The cancer involves the ductal system of the pancreas. The etiology includes age, prolong alcohol abuse, smoking, overweight, family history, and chronic pancreatitis (Goral, 2015). Genetic causes are also important for the development of the disease as mutated oncogenes have been detected in pancreatic cancer. Tumor markers such as CA 19−9 (carbohydrate antigen) and CEA

(carcinoembryonic antigen) are measured in the blood for diagnosis. Imaging methods such as endoscopic ultrasonography (EUS), magnetic resonance imaging (MRI) and computed tomography (CT), positron emission tomography (PET), endoscopic retrograde cholangiopancreatography (ERCP), magnetic resonance cholangiopancreatography (MRCP), and biopsy are used for diagnosis (Goral, 2015). The disease has fatal outcome. Management includes surgical resection, adjuvant chemotherapy, neoadjuvant therapy, and radiation therapy. There is an evolving role of synthetic protease inhibitors for therapeutic benefits in pancreatic cancer.

1.1.5 Ovarian cancer

Ovarian cancer is one of the commonest gynecological cancers seen in women, and it has high rate of mortality. The cancer is often diagnosed at a later stage; therefore, the diagnosis and treatment become more challenging. Increase in age, genetic causes, use of hormone replacement therapy, and history of cancer in the family are potential risk factors for ovarian cancer. Presence of tumor markers in the blood, ultrasound, CT, and PET scan may aid in the diagnosis.

1.2 Gastrointestinal cancers

1.2.1 Esophageal cancer

Esophageal cancer is the 11th commonest cancer in the world and accounts for the sixth most cancer with mortality (Global Burden of Disease Cancer Collaboration et al., 2019). Adenocarcinoma and squamous cell carcinomas are the two common types of esophageal cancers. Etiology for squamous cell carcinomas include alcohol and tobacco consumption, while abdominal obesity, gastroesophageal reflux, and Barret's esophagus are the major causes for adenocarcinomas (Yang et al., 2016). The chief complaint is dysphagia. Diagnosis is confirmed by biopsy, upper endoscopy, bronchoscopy, CT, PET, and MRI scan along with specific biomarkers. Surgical resection, chemotherapy, and radiotherapy form the basis of treatment.

1.2.2 Gastric cancer

Globally, gastric cancer is the fifth most common cancer and ranks as the third most in terms of cancer-related mortality (Smyth et al., 2020). The common causes include *Helicobacter pylori* infection and genetic polymorphism while smoking and alcohol consumption are considered as common risk factors. Anorexia, dyspepsia, and pain in the abdomen are common symptoms. Upper endoscopy, biopsy, presence of biomarkers, CT scan, and laparoscopy aid in the diagnosis. Surgical resection, chemotherapy, radiotherapy, and immunotherapy are helpful in treating gastric cancer cases.

1.2.3 Colon cancer

Colon cancer is considered to be the third most common cancer and the second most cause for all deaths related to cancer, globally (Duan et al., 2022). A diet with low fiber content, obesity, alcohol, and meat consumption are few of the risk factors. Symptoms such as change in bowel habits, bleeding from rectum, fatigue, and weight loss are present in a colon cancer

patient. Total blood count, presence of elevated carcinoembryonic antigen (CEA) in blood, ultrasound, CT, PET, MRI scans, colonoscopy, and biopsy aid in the diagnosis. There are different stages of the diseases according to the metastasis. Treatment includes colectomy, chemotherapy, and targeted therapy.

1.3 Proteolysis

Proteolysis is a pivotal biological catalytic process, primarily driven by a diverse group of enzymes known as proteases. These enzymes are ubiquitously distributed across various biological systems. In the realm of normal cellular function, proteases play indispensable roles in executing vital biological processes and exhibit regulatory control over a spectrum of cellular activities including gene expression, cellular differentiation, and programmed cell death (Egeblad and Werb, 2002). Nevertheless, contemporary research endeavors have unveiled the involvement of proteases in tumor initiation, growth, and advancement, evident in both primary tumor development and metastatic dissemination (Yang et al., 2009).

1.4 Types of proteases

To date, the human genome has unveiled approximately 990 distinct protease genes alongside 1605 genes encoding protease inhibitors (Rawlings et al., 2014). Proteases exhibit a diverse classification system based on the nature of the key amino acid present in their active sites and the mechanism employed for peptide bond cleavage. These classifications delineate proteases into six distinct groups: cysteine, serine, threonine, glutamic acid, aspartate, and matrix metalloproteases (Verbovšek et al., 2015).

Metastasis and the progression of tumors crucially depend upon the availability of nutrients and oxygen, a process orchestrated by a myriad of proteases present within the tumor microenvironment and neighboring tissues and organs (Yang et al., 2016). Numerous tumors have demonstrated elevated levels of proteases during their early stages, implicating these enzymes in multifaceted aspects of cancer biology including proliferation, modulation of immune responses, recruitment of inflammatory cells, facilitation of tumor invasion, angiogenesis, metastasis, apoptosis, and induction of epithelial to mesenchymal transition (Fig. 32.1). It is noteworthy that various types of proteases exhibit distinct and specific roles tailored to the unique characteristics of different cancer types. Protease inhibitors have garnered attention for their potential therapeutic role in cancer treatment. However, due to the fluctuating levels of different proteases throughout various stages of cancer development, a singular inhibitor cannot effectively target all classes of proteases (Fear et al., 2007). So researchers targeted identifying various types of protease and protease inhibitors in varied types of cancers.

Protease control the tumor growth by various processes. Angiogenesis is one of the processes. Protease inhibitors can be beneficial in checking the metastasis of the tumor.

Cysteine proteases (calpains) are distributed within cellular compartments such as the cytosol and lysosomes, with notable examples including cathepsins B, L, H, and S. Under pathological conditions, some cell types secrete these cysteine proteases. This enzyme family fulfills both general and specialized functions: general functions encompass protein catabolism, while specialized functions include the selective activation of extracellular protein degradation, modulation of macrophage activity, facilitation of bone resorption, and

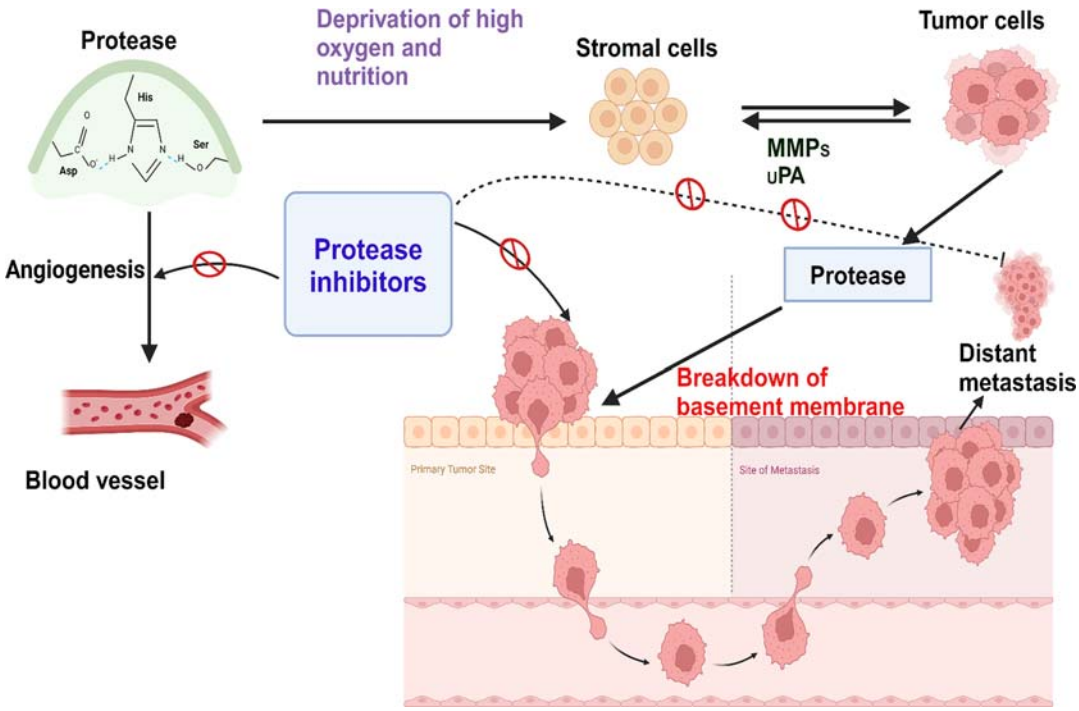

FIGURE 32.1 Role of proteases in pathogenesis of tumor. The schematic diagram shows the role of protease in tumor and how protease inhibitors can help in checking tumor growth and progression. Created with Biorender.com.

involvement in signaling pathways mediated by molecules such as interleukins, protein kinase C, and enkephalins (Zucker et al., 2000). Given their capability to degrade both intra- and extracellular matrix proteins (Gocheva and Joyce, 2007), cysteine proteases emerge as potential targets for anticancer therapeutic interventions.

Studies have shown that cysteine proteases, including cathepsins B, D, H, and L, are often upregulated in various cancers, such as pancreatic, colorectal, gastrointestinal, and many other carcinomas (Rakash, 2012; Kim et al., 1998; Mason and Joyce, 2011; Vasiljeva and Turk, 2008). Cathepsin B, a member of the cysteine protease family, has been implicated in the remodeling and degradation of basement membranes and connective tissues, thus exerting influence over processes critical to tumor growth, invasion, and metastasis (Joyce & Hahahan., 2004). Additionally, cathepsins serve as valuable prognostic markers in various cancers, including pancreatic, colorectal, breast, and tongue cancers (Hirai et al., 1999; Saleh et al., 2006; Kandalaft et al., 1993; Michl, 2012). Elevated levels of lysosomal proteases such as cathepsins D, H, and L have been observed in several cancer types (Fujise et al., 2000), with cathepsin L2 notably upregulated in melanomas, head and neck carcinomas, breast cancer, and gliomas (Lankelma et al., 2010). So cysteine protease can be used as prognosticate marker, and cysteine protease inhibitor is used as therapeutic intervention in a wide variety of cancers.

Cathepsin B levels tend to elevate across various endocrine cancers, particularly in pancreatic, ovarian, thyroid, and prostate cancers (Boy et al., 2008; Verbovšek et al., 2015; Egeblad

and Werb, 2002). Conversely, a general increase in cysteine cathepsins is observed in most cancer types (Rawlings et al., 2014).

In normal tissues and cells, endogenous cysteine protein inhibitors, such as cystatins, play a crucial role in controlling cathepsin activity, particularly cathepsin B, H, and L, which are lysosomal cysteine proteases. The cystatin superfamily encompasses a group of protease inhibitors capable of inhibiting multiple enzymes, including cathepsins B, H, and L (Ochieng and Chaudhuri, 2010).

Cancer types characterized by alterations in proteolytic pathways highlight the significant role of cysteine protease inhibitors (Turk et al., 1997). Cystatins, known for their inhibitory effects on tumor growth and metastasis, play a pivotal role in this context. Among the three types of cystatins, type I and II have been extensively studied, with type I often referred to as stefins A and B. Different types of proteases, protease inhibitors, and their implication in cancers were detailed in Table 32.1.

1.5 Studies related to pituitary cancer

Stefin A level or expression is well correlated with lower patients' survival, tumor progression, and also invasiveness. Immunological staining has revealed a significant decrease in stefin A levels in pituitary adenomas, coinciding with amplified levels of cathepsins (Strojnik et al., 2005). The elevated expression of cathepsins B and L in atypical adenomas indicates their potential involvement in tumor progression, as evidenced by increased proliferation and invasion. Moreover, increased levels of cathepsin B, specifically in functionally active adenomas, suggest its role in processing pituitary prohormones, influencing tumor behavior. Conversely, the downregulation of stefin A at an early stage in adenoma progression suggests its potential as a marker for early malignancy. Further investigation into these markers could offer valuable insights into the diagnosis and management of pituitary adenomas. In their study, Strojnik et al. (2005) investigated the immunohistochemical staining of cathepsins B and L, along with the inhibitor stefin A, in human hypophysis and pituitary adenomas. Their findings revealed higher immunohistochemical scores for

TABLE 32.1 Protease classes and cancer (endocrine and gastrointestinal).

Groups	Name of the protease	Cancer	References
Cysteine	Cathepsin B	Ovarian, pancreatic, thyroid	Verbovšek et al. (2015), Egeblad and Werb (2002)
Serine	Serpina1Alpha1 antitrypsin	Colorectal cancer	Liang et al. (2015), Pérez-Holanda et al. (2014)
Serine	Serpini2Pancpin	Pancreatic cancer	Ozaki et al. (1998)
Serine	SerpsinB13Hurpin/ Headpin	Ovarian cancer	Chang and Wu (2003), Wu et al. (2002)
MMPs	TMMPs—marimastat	Pancreatic cancer—gastroesophageal adenocarcinoma	Bramhall et al. (2001), Fenteany et al. (1995)

cathepsins B and L in atypical pituitary adenomas, indicating their significance in tumor progression. Additionally, they observed elevated cathepsin B levels in functionally active adenomas, irrespective of histology, supporting its role as a marker of neoplastic gland activity. Furthermore, the study highlighted the downregulation of stefin A, particularly in adenomas, suggesting its potential as a marker for early malignancy. This aligns with the proposed role of cathepsin B as a diagnostic marker for tumor progression, particularly in nonfunctional adenomas, aiding in the differentiation between benign and atypical tumors (Strojnik et al., 2005).

In the realm of serine proteases, approximately one-third of proteases are involved in the normal regulation of cellular and tissue physiology. However, dysregulated activity in these proteases can lead to pathological conditions such as cancer (DeClerck et al., 2004). Initially conceptualized as the "catalytic triad" consisting of Asp-His-Ser, serine proteases play crucial roles in various physiological processes.

Serpins, a group of proteins sharing a similar structure, were initially identified as a set of proteins capable of inhibiting serine proteases (Whisstock et al., 2010; Silverman et al., 2010). They also have a role as biomarkers in both diagnosis and prognosis of malignancy apart from treatment (Saleem et al., 2006).

1.5.1 Studies related to ovarian cancer

Epithelial ovarian cancer poses a significant clinical challenge, with high-grade invasive disease (TOV) exhibiting markedly lower survival rates compared to low malignant potential (LMP) disease. The progression from localized tumors to invasive metastasis involves intricate matrix proteolysis, a process regulated by protease inhibitors believed to play a pivotal role in restraining tumor advancement. Among the serine protease inhibitors identified through gene expression analysis, SERPINA1 emerged as a notable candidate, displaying distinct expression patterns between LMP and TOV serous tumors, validated through quantitative PCR (Q-PCR). Overexpression of SERPINA1 in invasive ovarian cancer cell lines impacted cell migration and invasion in vitro, indicating its potential influence on tumorigenic parameters. While no direct correlation was found between SERPINA1 immunostaining and patient survival, its differential localization in LMP and TOV tumors underscores its intricate involvement in ovarian cancer progression. Given the nuanced regulation of protein homeostasis by proteases and their inhibitors, further exploration of SERPINA1 is warranted to unravel its comprehensive impact on tumor behavior. In their study on epithelial ovarian cancer, Normandin et al. (2010) elucidated the differential expression of SERPINA1, a serine protease inhibitor, between low malignant potential (LMP) and high-grade invasive (TOV) tumors. They observed higher expression of SERPINA1 in LMP tumors compared with TOV tumors, with this disparity validated by quantitative PCR. Furthermore, they demonstrated the impact of SERPINA1 overexpression on cell migration and invasion in vitro, highlighting its potential role in modulating tumorigenic parameters. Although no direct association was found between SERPINA1 immunostaining and patient survival, the distinct localization of SERPINA1 in LMP and TOV tumors suggests its intricate involvement in ovarian cancer progression. This emphasizes the need for exploration of protease inhibitors such as SERPINA1 to understand their full impact on tumor behavior (Normandin et al., 2010).

1.5.2 Studies related to thyroid cancer

An earlier study postulated that SERPINE2 (also known as protease nexin-1) is upregulated in papillary thyroid carcinoma cells compared with normal thyroid cells (Chu et al., 2021). Upregulation is associated with high proliferative index and invasion (increased expression of MMP). In this study they observed the following: (1) Overexpression of SERPINE2 enhanced EGF and p-EGFR expression, which could be reversed by AG1478 and hence postulated SIRPINE2 by positive feedback loop interaction with epidermal growth factor (EGF), and its receptor (EGFR) signaling is the cellular mechanism in pathophysiology. (2) Silencing of SERPINE2 reduced cell proliferation as indicated by decreased expression of proliferation markers Ki67 and also suppressed cell migration and invasion accompanied by reduced expression of MMP2 and MMP9.

Serpine 2 and SLP1 are proteins with anticoagulant properties with also role in tumorigenesis (Stepień et al., 2017). The same study postulated the role of SERPINE 2 and Secretory leukocyte protease inhibitor (SLP1) in pathogenesis of papillary thyroid carcinoma (Stepień et al., 2017). In this study, they have measured both Serpine 2 and SLP1by ELISA, which were compared across papillary thyroid carcinoma, multinodular goiter, and healthy controls. This study showed markedly elevated concentrations of SERPINE2 and SLPI in patients with PTC in contrast to MNG and controls.

Further positive correlation was found between SERPINE2 and SLPI concentrations in PTC patients. Hence, both these molecules can help in differentiating papillary thyroid carcinoma among various thyroid diseases such as MNG and other malignant tumors, and also it may have a therapeutic role.

Molecular genetic studies have demonstrated that thyroid cancer, such as other malignant tumors, is associated with gradual accumulation of a series of genetic and epigenetic events, resulting in increased function of oncogenes and loss of function of tumor suppressor gene. Proteases play key roles in different biological processes, and their involvement in tumorigenesis and progression has attracted increased attention. Transmembrane protease serine 4 (TMPRSS4), a novel type II transmembrane serine protease (TTSP), was first identified in pancreatic cancer where its expression is strongly elevated.

Researchers demonstrated that TMPRSS4 is significantly overexpressed in papillary thyroid cancer compared with normal thyroid tissue (Jarzab et al., 2005). TMPRSS4 expression was significantly higher in malignant thyroid cancer than in benign (follicular adenoma) thyroid neoplasms and was an independent predictor of thyroid cancer (Guan et al., 2015). The enhanced activity of acid proteases in carcinoma, as observed in various in vitro experiments, may be accounted for by the accumulation of lysosomal enzymes and their unutilized proteolytic potential. Protease inhibitor inhibits local proliferation, enhances cell to cell signaling, and induces apoptosis by caspase related pathway. So it may be a promising therapeutic agent in future.

1.5.3 Studies related to pancreatic cancer

Ozaki et al. (1998) suggested that pancpin may function as a tumor suppressor gene in pancreatic cancer, similar to other serpins implicated in tumorogenesis. Hence, downregulation of pancpin expression by somatic mutations in pancreatic cancer indicates its potential role in cancer development and progression. It also has a role as a diagnostic marker or therapeutic target for pancreatic cancer.

1.5.4 Studies related to testicular cancer

Kazal-type serine protease inhibitor-2 (SPINK2) is a serine protease inhibitor, and the SPINK protein family has been shown to inhibit the expression of urokinase-type plasminogen activator (uPA). In addition, increased levels of uPA and the uPA receptor were observed in testicular cancer tissues. Protease inhibitor could be a promising way to prevent tissue invasion in testicular cancer (Shyu et al., 2019).

Matrix metalloproteinases (MMPs) constitute a group of nine or more homologous zinc endopeptidases that collectively degrade various components of the extracellular matrix (ECM), thus playing a crucial role in ECM remodeling and turnover. Elevated expression of MMPs is observed across multiple tumor types (Anastasov et al., 2011).

Pharmaceutical inhibitors targeting MMPs include batimastat and its chemical analog, marimastat. Marimastat has undergone extensive clinical trials, including studies in advanced pancreatic cancer. One trial aimed to discern differences in survival rates between varying doses of marimastat and conventional chemotherapy. While this trial failed to identify a survival benefit in the marimastat group, it did reveal that the highest dose of marimastat was equally effective as conventional therapy (Bramhall et al., 2001). In gastroesophageal adenocarcinoma, Bramhall et al. conducted a study administering marimastat with the goal of extending survival. They found that combining chemotherapy with marimastat resulted in increased survival benefits compared with chemotherapy alone (Fenteany et al., 1995). It has been observed that most of the protease inhibitors (MMP) increase the survival benefit, whereas the others are used as prognostic markers.

Serine proteases and MMPs are the subject of significant research because they appear to be associated to the process of tumor growth in pancreatic cancer. Several studies reported the inhibitory effects of protease inhibitor on pancreatic cancer cell invasion by directly antagonizing the activities of urokinase type plasminogen activator and tumor associated trypsinogen. Gabexate mesilate (synthetic serine protease inhibitor) suppressed not only the enzymatic activities of tumor-associated trypsinogen and urokinase-type plasminogen activator but also MMP-2 synthesis. Provided its capacity to hinder TNF-α-induced NF-κB initiation and to enhance apoptosis, gabexate mesilate may ultimately turn out to be important part of treatment for pancreatic cancer. Data from studies suggested that gabexate mesilate is possibly useful in treating the invasion, proliferation, and metastasis of pancreatic cancer.

Nafamostat is a serine protease inhibitor associated with inhibition of tumor progression in various tumors. Nafamostat improves treatment outcomes by blocking canonical NF-κB signaling and targeting TNFR1-stimulated caspase cleavage and mast cell tryptase activity. As a possible innovative anticancer drug, Nafamostat has been verified to improve cancer therapy resistance and prevent immunological resistance (Chen et al., 2019).

2. Other protease inhibitors implicated in endocrine cancers

2.1 Nelfinavir in different cancers

Nelfinavir (NFV), approved and widely utilized as an antiinfective agent for treating acquired immunodeficiency syndrome (AIDS), is also recognized for its anticancer properties demonstrated in both laboratory and animal studies. Its anticancer mechanism involves

the modulation of various cellular conditions, encompassing unfolded protein response, cell cycle regulation, apoptosis induction, autophagy inhibition, interference with the proteasome pathway, reduction of oxidative stress, alteration of the tumor microenvironment, and suppression of multidrug efflux pumps. In the following, we elaborate on studies highlighting NFV's efficacy across diverse cancer types.

2.2 In thyroid cancer

NFV, an HIV protease inhibitor, targets the PI3K/AKT and MAPK/ERK signaling pathways, which are crucial in thyroid cancers. Jensen et al. (2017) conducted a study investigating NFV's effects on cancer cells derived from follicular (FTC), papillary (PTC), and anaplastic (ATC) thyroid cancers. Through real-time microscopy and flow cytometry, NFV (10 μM) was found to prolong cell cycle progression and induce DNA fragmentation, indicative of its impact on cell proliferation and DNA damage. NFV treatment resulted in G0/G1 phase arrest, downregulation of cyclin D1 and CDK4, and activation of DNA damage markers γH2AX and p53BP1. Additionally, NFV (20 μM) triggered caspase-3 cleavage, suggesting apoptotic cell death across all tested cell lines. Notably, NFV decreased total and p-AKT levels in PTEN-deficient FTC133 cells, while it had no significant effect on total ERK and p-ERK in BRAF-positive BCPAP and SW1736 cells. Despite no alteration in EMT marker expression, NFV inhibited migration and reduced the survival of thyroid cancer cells in nonadherent conditions. These findings underscore NFV's potential as a therapeutic agent for thyroid cancer, based on its ability to inhibit proliferation and induce DNA damage observed in vitro.

2.3 In medullary thyroid cancer

Mutations in RET tyrosine kinase are implicated in the development of medullary thyroid cancer (MTC), with heat shock protein (HSP) 90 chaperone crucial for folding and stability of RET mutants. NFV, an HIV protease inhibitor, targets HSP90, presenting a potential therapeutic avenue. In a study by Kushchayeva et al. (2014), HSP90 overexpression was observed in 17 out of 36 human MTCs, correlating with metastases and RET mutations. Two MTC cell lines, TT and MZ-CRC-1, harboring C634W or M918T RET mutations, respectively, were treated with clinically relevant NFV concentrations. NFV (10 μM) significantly reduced viability by 55% and 10% in TT and MZ-CRC-1 cells, respectively. It downregulated RET protein levels, inhibited downstream targets (phosphorylated ERK, phosphorylated AKT, p70S6K/pS6), induced metabolic stress activating AMP-activated protein kinase, and increased autophagic flux. NFV also triggered mitochondrial membrane depolarization, oxidative stress, and DNA damage. Overall, the study demonstrated NFV's broad effectiveness against MTC cells, suggesting its potential as a novel therapeutic agent for MTC, leveraging its established safety profile as an anti-HIV medication.

2.4 In pancreatic cancer

Radiotherapy serves as a primary treatment option for inoperable locally advanced pancreatic cancer (LAPC), yet resistance to radiation is common, leading to disease progression and patient mortality. NFV has shown promise in preclinical studies by

enhancing radiation sensitivity through Akt downregulation (Gupta et al., 2005), reducing hypoxia (Pore et al., 2006) and improving tumor microvasculature. Brunner et al. (2008) conducted a phase I trial combining NFV with chemoradiotherapy in inoperable LAPC patients. Starting NFV 3 days before radiation therapy and chemotherapy (cisplatin and gemcitabine), 10 evaluable patients exhibited a 50% complete metabolic response in PET scans, with six patients undergoing secondary resection. Median overall survival was 18 months, accompanied by downregulation of p-Akt in peripheral blood mononuclear cells (PBMCs), and without significant additional toxicity. Subsequently, a phase II trial enrolled 23 LAPC patients with a life expectancy of at least 12 weeks, receiving NFV 1250 mg twice daily alongside radiotherapy and chemotherapy (cisplatin and gemcitabine). Results showed a median overall survival of 17.4 months and a 1-year survival rate of 73.4% (Wilson et al., 2016).

2.5 In rectal cancer

Hill et al. (2016) conducted a clinical trial involving 10 patients with advanced metastatic rectal cancer, where NFV (1250 mg twice daily) was combined with hypofractionated radiotherapy, omitting chemotherapy. MRI revealed tumor regression in five patients, while dynamic imaging (p-CT, DCI-MRI) suggested increased perfusion in the tumor area. In another study by Buijsen et al. (2013), eleven patients with locally advanced rectal cancer received NFV alongside standard radiotherapy and capecitabine. Three patients achieved a pathological complete response, and four others showed a major response. Diarrhea emerged as the most common adverse event, potentially linked to elevated plasma levels of NFV due to capecitabine's inhibition of CYP2C9, a metabolizing enzyme of NFV. The maximum tolerated dose of NFV was determined to be 750 mg twice daily.

2.6 In ovarian cancer

High-grade serous ovarian cancer (HGSOC) poses a significant threat to women worldwide, with platinum-based therapy constituting the traditional treatment approach. However, the emergence of platinum resistance leads to diminished life expectancy, necessitating the exploration of alternative therapies. Subeha et al. (2021) investigated the efficacy of NFV against HGSOC cells obtained from patients with varying platinum sensitivities. Regardless of platinum sensitivity, NFV exhibited a dose-dependent reduction in HGSOC cell number and viability, accompanied by an increase in hypodiploid DNA content. Additionally, NFV decreased clonogenic survival, indicating long-term residual damage. Phosphorylation of H2AX suggested NFV-induced DNA damage, linked to suppressed AKT and ERK pathways driving survival and proliferation signals. NFV also induced endoplasmic reticulum stress-related molecules, inhibiting protein synthesis and prompting cell death. This proapoptotic effect was underscored by increased phospho-eIF2α, ATF4, CHOP, Bax/Bcl-2 ratio, and cleaved caspase-7. Moreover, NFV enhanced both short-term cell cycle arrest and long-term toxicity induced by the proteasome inhibitor bortezomib. This in vitro study highlights NFV's potential to target HGSOC cells with varying platinum sensitivities through multiple mechanisms, suggesting its promising repurposing potential given its favorable safety profile.

3. Conclusion

One of the major challenges in the treatment of endocrine and gastrointestinal cancers is detection of the tumor at an advanced stage and the recurrence of the tumor. Protease is involved in tumor growth and progression. Tumor growth and metastasis depend on the nutrients and oxygen supply. Proteases are responsible for angiogenesis, cell proliferation, and tumor invasion. Abnormal regulation of proteases are associated with cancers. Protease inhibitors have shown in vitro and in vivo efficacy. Protease inhibitors are important in therapeutics for treatment of endocrine and gastrointestinal cancers.

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CHAPTER

33

Side effects of synthetic protease inhibitors used in gynecology cancers

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1. Introduction

1.1 Gynecological cancers

Gynecological cancers represent a formidable array of malignancies affecting various components of the female reproductive system, including the cervix, ovaries, uterus, fallopian tubes, vagina, and vulva (Parkash et al., 2023). These cancers constitute a significant health challenge globally, impacting women across diverse age groups. The World Health Organization (WHO) underscores their gravity by highlighting their substantial contribution to cancer-related mortality among women on a global scale. Indeed, the prevalence of gynecological cancers exhibits geographical disparities, reflecting differences in healthcare accessibility, screening protocols, and associated risk factors (Sung et al., 2021). Understanding the etiological underpinnings, risk determinants, early detection strategies, and treatment paradigms concerning gynecological cancers assumes paramount importance in the realm of healthcare. Such comprehension not only facilitates effective disease management but also holds the promise of ameliorating patient outcomes (Table 33.1).

The etiology of gynecological malignancies is multifaceted, involving a complex interplay of genetic, environmental, hormonal, and lifestyle factors. While the specific etiological pathways may vary across cancer types, several shared risk factors contribute to their development. Genetic anomalies, exemplified by mutations in genes like BRCA1 and BRCA2, escalate the susceptibility to ovarian and breast cancers, accentuating the role of genetic predisposition in certain cohorts (Kuchenbaecker et al., 2017). Human papillomavirus (HPV) infection, notably with high-risk strains, emerges as the predominant trigger for cervical cancer, underscoring the importance of HPV immunization and routine screening initiatives for

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TABLE 33.1 The major types of gynecological cancers.

Types of gynecological cancers	Tissue of origin
Cervical cancer	Cervical cancer, stemming from abnormalities in the cells lining the cervix, primarily manifests due to persistent infection with high-risk strains of the human papillomavirus (HPV). Early detection and intervention play pivotal roles in mitigating its impact. Screening methodologies, such as Pap smears and HPV testing, serve as indispensable tools in identifying precancerous or cancerous changes in cervical cells, thus enabling timely intervention and treatment (Burd, 2003).
Ovarian cancer	Ovarian cancer arises from the cells of the ovaries and frequently presents diagnostic challenges as it is often detected at advanced stages owing to nonspecific symptoms. The standard treatment protocol typically involves surgical resection followed by adjuvant chemotherapy. However, despite advancements in therapeutic strategies, the prognosis for patients with advanced-stage ovarian cancer remains largely unfavorable (Arora et al., 2023)
Uterine (endometrial) cancer	Endometrial cancer originates in the endometrium, the lining of the uterus, and is frequently linked to hormonal imbalances, obesity, and prolonged estrogen exposure. The cornerstone of its management involves surgical intervention, typically in the form of hysterectomy, with or without adjuvant therapy. This comprehensive approach aims to remove the cancerous tissue while addressing potential underlying risk factors and hormonal influences (Adefolaju et al., 2014; Mahdy et al., 2022).
Fallopian tube cancer	While comparatively rare, cancer can indeed originate from the fallopian tubes, often characterized by nonspecific symptoms and frequently diagnosed at an advanced stage. The primary treatment modalities for fallopian tube cancer typically involve surgical resection to remove the affected tissue, followed by adjuvant chemotherapy. Despite its rarity, prompt recognition and appropriate management are essential for optimizing patient outcomes in cases of fallopian tube cancer (Hundal et al., 2021).
Vaginal and vulvar cancer	Vaginal and vulvar cancers emerge from the tissues of the vagina and vulva, respectively. The management of these malignancies encompasses a multifaceted approach tailored to the individual's condition, including surgery, radiation therapy, and chemotherapy. The choice of treatment modality is contingent upon various factors, such as the stage and extent of the disease, with the aim of achieving optimal therapeutic outcomes while preserving quality of life (Rogers, 2022; Kaltenecker et al., 2023).

prevention (Cutts et al., 2007). Hormonal dynamics, encompassing imbalances such as early menarche, late menopause, and hormone replacement therapy, exert influence on the risk profiles of endometrial and ovarian cancers (Ignatov and Ortmann, 2020; Reid et al., 2017; Sullivan et al., 2016). Reproductive history similarly factors in, with variables such as nulliparity, infertility, and fertility drug usage impacting gynecological cancer susceptibility (Lundberg et al., 2019; Troisi et al., 2018). Moreover, lifestyle facets including smoking, obesity, dietary patterns, and exposure to specific carcinogens correlate with heightened risks of gynecological cancers (Rieck and Fiander, 2006).

The management of gynecological malignancies is tailored to the cancer subtype, stage, extent, and the patient's overall health status and treatment preferences. The cornerstone

therapeutic strategies (Ledermann et al., 2014) for gynecological cancers encompass the following:

- Surgery: Surgical resection stands as a cornerstone in the management of early-stage gynecological cancers, with the aim of excising the tumor and adjacent tissues. Minimally invasive techniques, such as laparoscopy and robotic-assisted surgery, offer advantages including reduced morbidity and quicker recovery times, enhancing patients' postoperative experiences.
- Chemotherapy: Chemotherapy assumes various roles in the treatment paradigm for gynecological cancers. It may be employed as adjuvant or neoadjuvant therapy to surgery, or as the primary treatment for advanced-stage or recurrent disease. Chemotherapy regimens are customized based on the specific type and stage of cancer, with the goal of targeting and eradicating cancer cells while minimizing systemic toxicity.
- Radiation therapy: Radiation therapy constitutes an integral component of treatment, particularly for gynecological cancers localized to the pelvic region. It can be administered externally or internally (brachytherapy), either as a primary treatment modality or in conjunction with surgery and chemotherapy. Radiation therapy aims to destroy cancer cells and prevent their proliferation while preserving surrounding healthy tissues.
- Targeted therapy: Targeted therapies have revolutionized the landscape of cancer treatment, offering more precise and selective approaches to combating malignancies. In the context of gynecological cancers, PARP inhibitors have emerged as a promising therapeutic option, particularly for BRCA-mutated ovarian cancers. By specifically targeting cancer cells while sparing normal tissues, targeted therapies offer enhanced efficacy and reduced toxicity compared with conventional chemotherapy.
- Immunotherapy: Immunotherapy represents a cutting-edge approach that harnesses the body's immune system to recognize and eliminate cancer cells. While still in the investigational phase for many gynecological cancers, immune checkpoint inhibitors have shown promise as potential treatment options, particularly in cases of refractory or metastatic disease. Immunotherapy holds the potential to revolutionize the treatment landscape by offering durable responses and improved outcomes for patients with gynecological cancers.

1.2 Basic science of protease inhibitors: Mechanism of action

Protease inhibitors operate at the molecular frontier of cancer therapy. These compounds are designed to specifically target proteases—enzymes that play a pivotal role in protein degradation and turnover. In the context of cancer, proteases are instrumental in tumor growth, angiogenesis, and metastasis (Vizovisek et al., 2021). They facilitate the breakdown of extracellular matrix components, enabling cancer cells to invade and spread. By inhibiting these enzymes, protease inhibitors disrupt these critical pathways, essentially impeding the growth and dissemination of tumors (Rudzińska et al., 2021).

The efficacy of these inhibitors lies in their specificity and potency. They are engineered to bind to the active sites of target proteases, thereby blocking their catalytic activity

(Rudzińska et al., 2021). This targeted approach not only curtails tumor progression but also minimizes damage to healthy tissues, a significant advancement over traditional chemotherapeutic agents.

1.3 Evolution and development of synthetic protease inhibitors in oncology

The journey of synthetic protease inhibitors in oncology is a testament to the relentless pursuit of precision medicine. Initially, these inhibitors were explored in the context of HIV therapy, where their success in targeting viral proteases paved the way for cancer applications (Adefolaju et al., 2014). The realization that certain proteases are overexpressed or aberrantly activated in various cancers spurred intensive research into developing inhibitors specific to these proteases.

Over the years, the development of synthetic protease inhibitors has evolved dramatically. Early generations of these inhibitors faced challenges such as poor selectivity and bioavailability. However, with advancements in molecular biology and drug design, newer generations have overcome many of these hurdles. Today, these inhibitors are more specific, potent, and less toxic, making them a mainstay in the treatment of several cancers, including those of the gynecological malignancies.

Proteases, which are enzymes that have crucial functions in various cellular processes such as protein degradation, cell signaling, and tissue remodeling, have led to the emergence of synthetic protease inhibitors as valuable tools in cancer therapy (Park et al., 2020). The development of these inhibitors has been motivated by a profound understanding of protease functions and their involvement in the progression of cancer. These inhibitors are specifically designed to target and obstruct the activity of particular proteases that are implicated in the growth of tumors, metastasis, and angiogenesis (Rudzińska et al., 2021). There are different classes of synthetic protease inhibitors (Fear et al., 2007).

- Serine protease inhibitors: These compounds effectively inhibit serine proteases such as trypsin, chymotrypsin, and plasmin. Due to their roles in tumor invasion and angiogenesis, they hold potential applications in cancer treatment (Mkaouar et al., 2019).
- Cysteine protease inhibitors: These inhibitors specifically target cysteine proteases such as cathepsins. Cathepsins are involved in tumor invasion, metastasis, and resistance to apoptosis (Siklos et al., 2015).
- Matrix metalloproteinase (MMP) inhibitors: MMPs play a significant role in remodeling the extracellular matrix and tumor invasion. Synthetic inhibitors aim to regulate their activity (Cabral-Pacheco et al., 2020).
- Aspartic protease inhibitors: These inhibitors are designed to target aspartic proteases such as HIV protease. Although primarily used in antiviral therapy, they may also have implications in cancer research (Subeha and Telleria, 2020).

1.4 Protease inhibitors for the treatment of gynecological cancers

Despite strides in early detection and therapy, gynecological malignancies persist as a challenge due to their diverse nature, frequent recurrences, and limited options for advanced-stage cases. A promising avenue lies in protease inhibitors, a novel therapeutic modality

demonstrating potential in combating these cancers. By targeting pivotal enzymes in tumor progression and metastasis, protease inhibitors present an appealing option for combination therapies, aiming to enhance patient outcomes (Rudzińska et al., 2021).

Preclinical investigations exploring protease inhibitors' efficacy in gynecological cancers yield encouraging findings. Studies utilizing cellular models and animal subjects reveal the inhibitors' ability to curb tumor proliferation, invasion, and vascularization across various gynecological cancer subtypes. Moreover, they exhibit a capacity to heighten gynecological cancer cell sensitivity to conventional chemotherapy and radiation, suggesting their utility in combination regimens (Bandiera et al., 2016; Drubin et al., 2006; Kumar et al., 2009; Mehner et al., 2015; Park et al., 2020).

While preclinical data furnish valuable insights, clinical validation of protease inhibitors in gynecological cancers is ongoing. Several trials assess the safety and efficacy of these inhibitors, either as monotherapy or adjuncts to standard treatments, particularly in refractory or advanced cases. Although preliminary, trial results hint at the promise of protease inhibitors in augmenting therapeutic outcomes for gynecological cancers.

Despite their therapeutic potential, protease inhibitors entail side effects pivotal for patient care. These encompass a spectrum from mild to severe, including gastrointestinal disturbances such as nausea, vomiting, and diarrhea, commonly manageable with supportive measures (Altun and Sonkaya, 2018). Hematological effects such as anemia and thrombocytopenia necessitate vigilant monitoring and intervention (Vitzthum et al., 2020).

Unique to protease inhibitors are risks of hepatotoxicity and cardiovascular events, mandating regular liver function tests and cardiac surveillance (Bruder-Nascimento et al., 2020; Sulkowski et al., 2000). Additionally, patients may manifest drug-specific reactions such as skin rashes or hypersensitivity (Corbett et al., 2002). Notably, prolonged usage may culminate in cumulative toxicities, emphasizing the necessity for continual assessment and management of side effects throughout treatment.

1.5 Types of protease inhibitors for the treatment of gynecological cancers

The pursuit of protease inhibitors for gynecological cancer therapy stands as a dynamic field of inquiry, with relentless endeavors aimed at unraveling their mechanisms, refining formulations, and pinpointing predictive response markers. Concurrently, the investigation into combination regimens, integrating protease inhibitors with targeted therapies, immunomodulators, or traditional cytotoxic interventions, presents a compelling avenue for enhancing treatment efficacy and circumventing resistance in gynecological malignancies. The spectrum of protease inhibitors under scrutiny in preclinical and clinical investigations is outlined in Fig. 33.1 and Table 33.2, delineating their diverse therapeutic potentials and avenues for exploration.

These protease inhibitor drugs are used to treat various gynecological cancers.

1. Specific synthetic protease inhibitors in gynecological cancer

In the realm of gynecological oncology, the emergence of synthetic protease inhibitors has heralded a new era of targeted therapy, offering a beacon of hope for patients grappling with these formidable malignancies. This section elucidates the specific synthetic protease inhibitors employed in gynecological cancers, detailing their indications and therapeutic benefits.

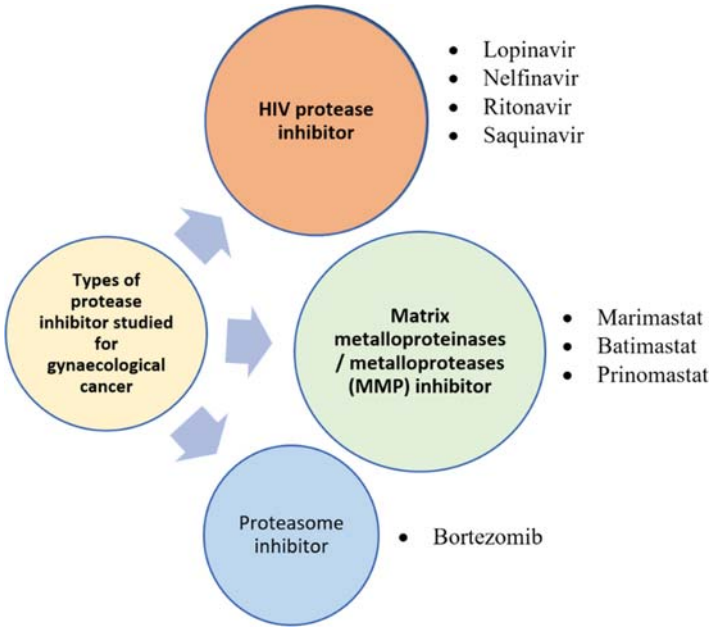

FIGURE 33.1 Classes of proteases inhibitors being studied as treatment for gynecological cancers. The figure shows different protease inhibitors with specific names of drugs. *Created with Biorender.com*.

1.5.1 Protease inhibitors used in preclinical studies

Synthetic protease inhibitors have established themselves as pivotal players in gynecological cancer therapeutics, stepping in where conventional chemotherapeutic agents have encountered limitations. Engineered to selectively engage key proteases implicated in cancer advancement, these inhibitors have wrought a paradigm shift in treatment strategies. A compilation of select protease inhibitors scrutinized in preclinical investigations for gynecological cancer management is encapsulated in Table 33.2, underscoring their potential contributions to therapeutic innovation.

1.5.2 Protease inhibitors used in clinical trial

Clinical trials have borne witness to the advent of protease inhibitors in the realm of gynecological cancer therapeutics. Table 33.3 delineates the protease inhibitors that have traversed into clinical evaluation, signifying a significant stride in therapeutic advancement. Meanwhile, Fig. 33.2 offers a visual representation of the diverse array of protease inhibitors deployed in the treatment landscape of gynecological malignancies, elucidating their respective mechanisms of action.

These are protease inhibitors that are useful in treatment of various gynecological cancers. The names of the drugs are mentioned.

TABLE 33.2 List of some protease inhibitors investigated in preclinical studies for the treatment of gynecological cancers.

Types of protease inhibitor	Description
Marimastat	Marimastat, a broad-spectrum matrix metalloproteinase (MMP) inhibitor, has undergone scrutiny in preclinical investigations for its envisaged antitumor efficacy in gynecological malignancies, encompassing ovarian and cervical cancers (Ile, 2007).
Batimastat	Batimastat, an additional MMP inhibitor, has undergone preclinical evaluation for its potential in impeding tumor proliferation and metastasis in gynecological cancers (Burke et al., 1997; Memtsas et al., 2009).
Prinomastat	Prinomastat, a discerning inhibitor of MMP-2, has been scrutinized in preclinical investigations for its potential antitumor effects specifically in ovarian cancer (Hande et al., 2004).
Lopinavir	Lopinavir, an alternative HIV protease inhibitor, has been the subject of preclinical scrutiny due to its observed antiproliferative and proapoptotic properties in cervical cancer cell lines (Adefolaju et al., 2014).
Nelfinavir	Nelfinavir, a protease inhibitor conventionally employed in HIV/AIDS treatment, has garnered attention in preclinical investigations for its prospective anticancer properties. These studies have indicated its capacity to impede proliferation and trigger apoptosis in ovarian cancer cells (Garcia-Soto et al., 2021).
Ritonavir	Ritonavir, a well-known protease inhibitor utilized in the management of HIV (human immunodeficiency virus), has been the focus of preclinical inquiries regarding its prospective utility in the treatment of ovarian and cervical cancer cells (Banno et al., 2015; Barillari et al., 2012).
Saquinavir	Saquinavir, primarily employed to suppress the activity of the HIV protease enzyme, has demonstrated inhibitory effects on uterine cervical intraepithelial neoplasia (CIN) cell invasion. Additionally, it has been noted to decrease the expression and proteolytic activity of MMP-2 and MMP-9 in treated cells sourced from CIN or cervical carcinoma lesions (Barillari et al., 2012).
Bortezomib	Bortezomib, an approved proteasome inhibitor for multiple myeloma and mantle cell lymphoma, has exhibited promising potential in preclinical investigations for gynecological cancers. These studies have highlighted its ability to induce apoptosis and inhibit angiogenesis, suggesting a therapeutic role in this context (Kao et al., 2014; Lee et al., 1949).

1.6 General overview of common side effects in cancer treatment

While synthetic protease inhibitors represent a breakthrough in cancer therapy, they are not devoid of side effects. Understanding these side effects is crucial, as they can significantly impact patient quality of life and treatment compliance. Common side effects in cancer treatments, including those caused by protease inhibitors, range from mild to severe. Patients may experience gastrointestinal disturbances such as nausea, vomiting, and diarrhea, often manageable with supportive care (Altun and Sonkaya, 2018; Sherman and Fish, 2000). Hematological side effects such as anemia and thrombocytopenia are also prevalent, requiring careful monitoring and intervention (Jameus et al., 2021).

More specific to protease inhibitors are the risks of hepatotoxicity and cardiovascular events, necessitating regular liver function tests and cardiovascular monitoring (Hatleberg

TABLE 33.3 List of some protease inhibitors that have entered clinical trials of gynecological cancers.

Bikunin	**Bikunin**, also known as **urinary trypsin inhibitor (UTI)**, is a small chondroitin sulfate proteoglycan (PG) with remarkable properties (Pugia et al., 2007). Bikunin plays a crucial role in stabilizing the ECM of connective tissues. This function contributes to tissue integrity and homeostasis. It functions as an antimetastatic drug by inhibiting the urokinase-type plasminogen activator (uPA) and its receptor (uPAR), which mediate tumor invasiveness through a downregulation of uPA mRNA expression (Lepedda et al., 2023).
Nelfinavir	In both in vivo and in vitro models, it has been demonstrated that nelfinavir (NFV), an oral human immunodeficiency virus 1 (HIV-1) protease inhibitor, radiosensitizes pancreatic tumors unrelated to HPV as well as head and neck squamous cell carcinomas (HNSCCs) (Garcia-Soto et al., 2021)
Lopimune (lopinavir/ ritonavir)	Lopimune is an HIV protease inhibitor and is active against HIV-1 and HIV-2 (Boyd et al., 2013). It works by halting HIV-1's development, which prevents the virus from spreading. Thus, lopinavir's primary antiviral effect is to stop vulnerable cells from being infected again (Cvetkovic and Goa, 2003). However, there are reports that speculated that HIV protease inhibitors (PIs) may have activity against HPV related cervical disease (Hampson et al., 2016).
Bortezomib and pegylated liposomal doxorubicin (PLD)	The US FDA has authorized bortezomib as the first proteasome inhibitor for the treatment of mantle cell lymphoma, relapsed/refractory multiple myeloma, and newly diagnosed multiple myeloma (Kane et al., 2003). Malignant cells may undergo programmed cell death as a result of proteasome inhibition, which may encourage the breakdown of antiapoptotic proteins and limit the degradation of proapoptotic proteins (Chen et al., 2011). It has entered phase II clinical trial for ovarian cancer (Lee et al., 1949).
Prinomastat	**Prinamostat**, also known by its generic name **pracinostat**, is an **investigational drug** with potential therapeutic applications. It is a member of the sulfonamide class of MMP inhibitors (Weinmann and Ottow, 2006). The extracellular matrix is broken down by the MMP family of secreted or transmembrane zinc-dependent enzymes under a variety of normal and pathological circumstances. MMPs are important for tumor development, invasion, and angiogenesis in cancers (Scatena, 2000).

et al., 2021). Additionally, patients may develop drug-specific reactions, such as skin rashes or hypersensitivity. Importantly, the long-term use of these agents can lead to cumulative toxicities, underscoring the need for ongoing assessment and management of side effects throughout the treatment course (Lee et al., 2023).

In summary, synthetic protease inhibitors have revolutionized the treatment of gynecological cancers, offering targeted and effective intervention. Their development reflects a remarkable journey of scientific innovation, driven by a deeper understanding of cancer biology. However, the management of their side effects remains a critical component of patient care, balancing the benefits of these powerful agents with the necessity of preserving patient well-being. As research continues to evolve, the future of cancer therapy lies in further refining these treatments, enhancing their efficacy while minimizing their adverse impacts.

1.7 Side effects of protease inhibitors in gynecological cancers treatment

The advent of synthetic protease inhibitors in the treatment of gynecological cancers has been a significant milestone, offering targeted and effective therapeutic options. However,

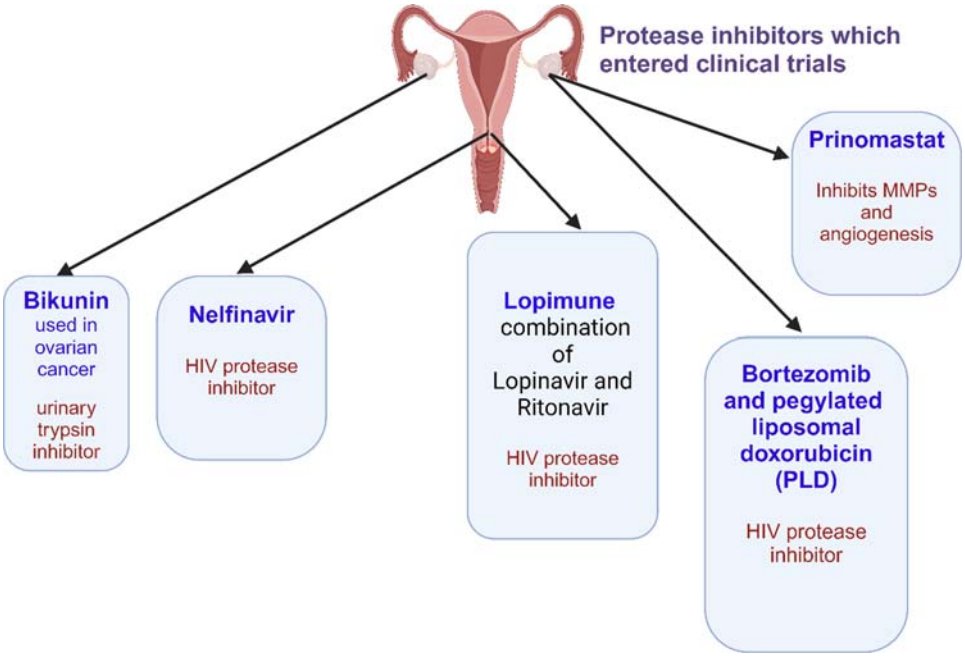

FIGURE 33.2 Schematic diagram showing various protease inhibitors that have entered clinical trials. Protease inhibitors in different gynecological cancers. *Created with Biorender.com*.

an in-depth understanding of their individual side effects is crucial for optimizing patient care and outcomes. The side effects of protease inhibitors used in clinical trials were tabulated in Table 33.4.

1.8 Classification of side effects

While the efficacy of synthetic protease inhibitors is noteworthy, understanding and managing their side effects is paramount. These side effects are diverse, ranging from mild symptoms such as nausea and fatigue to severe complications such as increased bleeding risks and cardiovascular events. Classifying these side effects into acute, chronic, and long-term categories aids in better understanding and management. Acute side effects often occur shortly after treatment initiation and are generally reversible. Chronic side effects develop over time and can be more severe, requiring ongoing management. Long-term side effects may emerge months or years after treatment and include complications such as cardiovascular issues and secondary malignancies.

1.8.1 Acute side effects

These occur shortly after the initiation of therapy and are usually reversible. Common acute side effects include nausea, vomiting, diarrhea, and skin reactions (Giri et al., 2020). For instance, patients receiving bortezomib and pegylated liposomal doxorubicin (PLD) may experience gastrointestinal disturbances as their body adjusts to the medication (Lee et al., 1949).

TABLE 33.4 Clinical trials on protease inhibitors used in gynecology cancers.

Title of article (author, year)	Types of protease inhibitor	Types of gynecological cancer	Types and description of study	Reported side effects
Therapeutic efficacy of once-daily oral administration of a Kunitz-type protease inhibitor, bikunin, in a mouse model and in human cancer (Kobayashi et al., 2004)	Bikunin	Advanced uterine cervical carcinoma	A phase I clinical trial was conducted to ascertain the maximum-tolerated dose (MTD) and safety profile of a once-daily oral administration regimen. The trial focused on patients diagnosed with locally advanced uterine cervical carcinoma following definitive treatment. Nine patients were enrolled, and an escalating dose of bikunin was orally administered over a 7-day period. The doses administered sequentially were 3 mg/kg per day, followed by 10 mg/kg per day, and finally 30 mg/kg per day. The primary objectives of the trial were to establish the MTD of bikunin and evaluate its safety profile in this patient population.	• No dose-limiting toxicities. • MTD of the bikunin schedule was not defined. • The clinical toxicity observed during therapy at dose level 3 (30-mg/kg) consisted of dose-related nausea (1 patient) and diarrhea (1 patient) occurring within 4–5 h after dosing. • Limited to short-term, acute side effects that resolved spontaneously by 24 h.
Phase 1 trial of nelfinavir added to standard cisplatin chemotherapy with concurrent pelvic radiation for locally advanced cervical cancer (Garcia-Soto et al., 2021)	Nelfinavir	Carcinoma of the cervix	Thirteen patients diagnosed with advanced cervical cancer were enrolled in a study where they received oral nelfinavir (NFV) twice daily. The treatment commenced at a dose of 875 mg (designated as dose level 1, DL1) 1 week prior to initiating cisplatin-based chemoradiation (CRT). Throughout the 7-week course of cisplatin and pelvic external-beam radiation therapy (PEBRT) treatment with brachytherapy, patients continued to receive NFV at this initial dose level. If the treatment was well tolerated, the dose of NFV was escalated to 1250 mg twice daily (designated as DL2) according to the standard dose-escalation protocol.	Side effects for DL1: • 1 patient had grade 4 hypokalemia and hypomagnesemia. • 1 patient had 1 day severe vomiting. • 1 patient with lymphopenia of grade 4 and 3 patients with grade 3. • 1 patient suffered from grade 3 urinary obstruction and thromboembolism. Side effects for DL2: • 1 patient had grade 3 nausea and ileus. • 1 patient had grade 3 diarrhea. • 1 patient had impaired renal function with grade 3 hematuria.
A single-arm, proof-of-concept trial of lopimune (lopinavir/ritonavir) as a treatment for HPV-related preinvasive cervical disease (Hampson et al., 2016)	Lopimune (lopinavir/ritonavir)	HIV-negative women diagnosed as high-risk HPV positive with high-grade squamous intraepithelial lesions (HSIL)	A single-arm proof-of-concept trial was conducted involving 23 HIV-negative women diagnosed with high-risk HPV positivity and high-grade squamous intraepithelial lesions (HSIL). These participants were provided with lopimune soft gel capsules containing 133 mg lopinavir and 33 mg ritonavir, manufactured by CIPLA, India. The capsules were intended for vaginal insertion, with participants instructed to use one capsule once or twice daily for a maximum period of 2 weeks. Throughout the 2-week duration of lopimune therapy, participants were scheduled for two additional visits per week. During these visits, a structured questionnaire was administered to assess adverse drug reactions and drug compliance. Additionally, physical examinations were conducted to monitor the participants' response to the therapy.	• Minor side effects reported include nausea (8.7%), headache (13%) followed by vaginal irritation (8.7%); feeling faint/dizziness (8.7%). • Abnormal vaginal discharge (8.7%) and abdominal pains (4.3%). • No serious adverse reactions or drug events • Such as death, life-threatening events, hospitalization, or disability observed. • None of the study subjects reported, vaginal dryness, bleeding/spotting, painful intercourse, palpitations, unusual weakness, vomiting, decreased appetite, diarrhea, pale stools, itchy skin, skin rashes, increased thirst, increased frequency of urination, visual disturbance, chest pains, or jaundice.

TABLE 33.4 Clinical trials on protease inhibitors used in gynecology cancers.—cont'd

Title of article (author, year)	Types of protease inhibitor	Types of gynecological cancer	Types and description of study	Reported side effects
A phase II trial to evaluate the efficacy of bortezomib and liposomal doxorubicin in patients with BRCA wild-type platinum-resistant recurrent ovarian cancer (KGOG 3044/EBLIN) (Y. J. Lee et al., 2022)	Bortezomib and pegylated liposomal doxorubicin (PLD)	Ovarian cancer	A phase II single-arm multicenter trial was conducted on women aged 19 years and above who were diagnosed with platinum-resistant or refractory recurrent ovarian cancer and had a wild-type BRCA status. Platinum resistance was defined as disease progression within 6 months after the last platinum-based chemotherapy, while refractory disease was defined as progression within 1 month after the last platinum-based chemotherapy. In this trial, patients received bortezomib at a dose of 1.3 mg/m^2 administered as a subcutaneous injection on days 1, 4, 8, and 11 of each 4-week cycle. Additionally, patients received pegylated liposomal doxorubicin (PLD) at a dose of 40 mg/m^2 via intravenous infusion, administered over a period of more than 60 min, specifically on day 4 of each cycle. The treatment regimen aimed to assess the efficacy and safety of this combination therapy in patients with recurrent ovarian cancer who exhibited platinum resistance or refractoriness and had a wild-type BRCA status.	• The most frequent adverse effects were neutropenia, nausea, and dermatologic toxicities, which were predominantly of low grade. • Grade 3 or higher-grade hematological toxicities (4.3% neutropenia and 4.3% thrombocytopenia) were generally consistent with those reported in previous studies in ovarian cancer (7% neutropenia and 10.3% thrombocytopenia). • Gastrointestinal complaints were the most frequently reported grade 3/4 adverse effects. • Peripheral neuropathy of bortezomib was reported in 8.7% of patients. • Grade 3 mucositis was experienced by one patient.
Phase I and pharmacokinetic study of prinomastat, a matrix metalloprotease inhibitor (Hande et al., 2004)	Prinomastat	Ovarian cancer (3 patients) and more than 9 types of other cancers	A phase I trial enrolled 75 patients aged at least 18 years diagnosed with confirmed advanced cancer for which no satisfactory treatment was available at the time of enrollment. Prinomastat was administered in tablet form, with doses ranging from 1 to 50 mg, twice daily at approximately 12-hour intervals, to be taken before meals on an empty stomach. The trial aimed to assess the safety, tolerability, and pharmacokinetics of prinomastat in this patient population.	• For patients who received 5 mg of prinomastat twice daily, grade 3 fatigue was recorded in 1 patient and another 1 patient experienced grade 1 arthralgia. • Grade 2 or 3 adverse, drug-related events that occurred in at least 5% of patients included arthralgias (23% of patients), fatigue (11%), pain in limb (8%), and myalgia (5%) • The most advanced effects (i.e., contractures of fingers, limited range of motion of the shoulders, and palmar nodules), all occurred among patients receiving prinomastat doses >25 mg twice daily. • Other grade ≥2 adverse events occurring in 1 or 2 patients each included anorexia, localized or generalized pain, weakness, constipation, dermatitis, facial edema, skin hypertrophy, taste disturbance, and urticaria. • The most frequent hematologic abnormalities observed as grade 1/2 are increased in blood alkaline phosphatase, aspartate aminotransferase, prothrombin time, anemia, and hypocalcemia. • 4 patients had grade 3 increased of total bilirubin.

1.8.2 Chronic side effects

These are persistent side effects that develop over time and can range from mild to severe. Chronic toxicities often include hematological complications such as anemia, neutropenia, and thrombocytopenia. Prinomastat, used in the treatment of ovarian cancers, is associated with hematologic disturbances, requiring ongoing monitoring and management (Hande et al., 2004).

Long-term side effects: Long-term or late-emerging side effects can manifest months or years after treatment. These may include cardiovascular complications, secondary malignancies, and potential impacts on fertility (Nurgali et al., 2018). The prolonged inhibition of proteases can lead to cumulative effects on normal tissue function, necessitating vigilance even after the completion of therapy.

1.9 Exploration of common and rare side effects

The spectrum of side effects ranges from common, often manageable symptoms to rare, potentially life-threatening conditions. For instance, lopimune, widely used in ovarian cancer, commonly causes fatigue, headache, and dizziness (Hampson et al., 2016). However, no serious adverse side effect were reported. Bortezomib and pegylated liposomal doxorubicin (PLD), while effective in starving tumors, can lead to rare but serious side effects such as hematological toxicities (Lee et al., 1949). The management of these side effects is a critical component of patient care. It involves a multidisciplinary approach, encompassing dose adjustments, supportive medications, and in some cases, discontinuation of the offending agent.

1.10 Mechanism behind side effects

The introduction of synthetic protease inhibitors in the treatment of gynecological cancers marked a turning point in oncological therapeutics. These agents, while transformative in their efficacy, bring with them a constellation of side effects whose underlying mechanisms warrant thorough exploration. This section delves into the biological basis of these side effects, the interaction of these inhibitors with other medications, and the role of genetic predisposition and patient-specific risk factors in their manifestation.

1.10 1 Biological basis of side effects

Synthetic protease inhibitors, designed to target specific enzymes crucial in cancer cell proliferation and metastasis, can inadvertently affect normal physiological processes. The proteases they inhibit, while overexpressed in cancer cells, are also integral to normal cell functions. Inhibiting these enzymes disrupts not only cancerous pathways but also essential biological processes, leading to a range of side effects (Rudzińska et al., 2021).

For example, prinomastat is used in ovarian cancer, important for tumor development, invasion, and angiogenesis in cancers. While they are effective in reducing tumor size, they can also impact normal cells' ability to function and cause myalgia and arthralgias (Hande et al., 2004). Similarly, bevacizumab's mechanism of inhibiting angiogenesis, although effective in starving tumors, can impair normal blood vessel formation, leading to hypertension and increased risk of bleeding.

1. Interaction with other medications and impact on different body systems

The interplay between synthetic protease inhibitors and other medications adds another layer of complexity to their side effect profile. These inhibitors can either potentiate or diminish the effects of concurrent medications through various pharmacokinetic and pharmacodynamic interactions. For instance, protease inhibitors may alter the metabolism of coadministered drugs, leading to increased toxicity or reduced efficacy (Svedmyr et al., 2022).

Moreover, the systemic nature of these inhibitors means their effects are not confined to the tumor alone. They can impact multiple body systems, manifesting as a wide array of side effects. Gastrointestinal disturbances, hepatotoxicity, and cardiovascular effects are among the most common system-wide side effects (Maksimovic-Ivanic et al., 2017). This multisystem impact necessitates a holistic approach to patient management, addressing each affected system's unique needs.

1. Genetic predisposition and patient-specific risk factors

The variability in side effect profiles among patients can often be attributed to genetic predispositions and individual risk factors. Genetic variations, particularly those affecting drug metabolism and DNA repair mechanisms, can significantly influence a patient's response to these inhibitors (Karczewski et al., 2012). For example, individuals with certain polymorphisms in genes encoding drug-metabolizing enzymes may experience increased toxicity or reduced efficacy of protease inhibitors (Ashton et al., 2007).

Furthermore, patient-specific factors such as age, comorbidities, and previous treatment history play a pivotal role in the manifestation and severity of side effects (Falzone et al., 2018). Older patients or those with preexisting liver or kidney conditions may be more susceptible to the toxicities of these agents. Similarly, patients with a history of hematological diseases may be at higher risk for the cardiac side effects of agents such as prinomastat (Hande et al., 2004).

In conclusion, understanding the side effects of synthetic protease inhibitors in gynecological cancers requires a multifaceted approach, considering the biological mechanisms, drug interactions, and individual patient factors. This knowledge is instrumental in devising personalized treatment strategies, aiming to maximize therapeutic benefits while minimizing adverse effects. As research progresses, it is hoped that this understanding will lead to the development of more selective inhibitors with fewer off-target effects, further revolutionizing cancer care and improving patient outcomes.

1.11 Management of side effects

In the landscape of gynecological cancer treatment, synthetic protease inhibitors stand as pivotal agents, offering new therapeutic avenues. However, their potential side effects necessitate a comprehensive management strategy to optimize patient outcomes and maintain quality of life. This section delves into the multifaceted approach for managing these side effects, encompassing strategies for prevention and early detection, pharmacological and non-pharmacological interventions, and the indispensable role of patient education and counseling.

1. Strategies for prevention and early detection of side effects

Effective management of side effects begins with proactive prevention and early detection. This involves a thorough assessment of patient history and potential risk factors before initiating treatment. Tailoring the choice and dosage of protease inhibitors based on individual patient profiles can significantly reduce the risk of adverse effects (Svedmyr et al., 2022). Regular monitoring throughout the treatment course is crucial. This includes routine blood tests, liver and kidney function tests, and cardiovascular monitoring, depending on the specific inhibitor and patient's baseline health status (Svedmyr et al., 2022).

For instance, in patients receiving prinomastat, regular monitoring of blood counts can preemptively identify hematological toxicities, allowing for timely intervention (Hande et al., 2004). Similarly, for bortezomib and pegylated liposomal doxorubicin (PLD), monitoring blood can detect early signs of neutropenia and thrombocytopenia, respectively (Lee et al., 1949).

1.11.1 Pharmacological and nonpharmacological management approaches

Once side effects emerge, a combination of pharmacological and nonpharmacological approaches is often required for effective management.

1.11.1.1 Pharmacological interventions

These involve the use of medications to either treat the side effects directly or mitigate their impact. For gastrointestinal toxicities, antiemetics and antidiarrheal agents can be prescribed (Akbarali et al., 2022). Hematological side effects may require growth factors or blood transfusions, and cardioprotective medications can be employed for cardiovascular side effects (Hatleberg et al., 2021). Importantly, dose adjustments of the protease inhibitor itself can be a critical tool in managing toxicity.

1.11.1.2 Nonpharmacological interventions

Lifestyle modifications, dietary changes, and supportive therapies play a significant role in managing side effects (Jia et al., 2022). For example, dietary interventions can help manage gastrointestinal symptoms, while physical therapy and exercise can aid in combating fatigue and maintaining cardiovascular health. Psychological support and complementary therapies such as acupuncture may also be beneficial in addressing the overall well-being of the patient (Birch, 2019).

Patient education and counseling are cornerstone elements in the management of side effects. Educating patients about the potential side effects, their signs and symptoms, and when to seek medical attention is vital (Paterick et al., 2017). It empowers patients to be active participants in their care and enhances their ability to recognize and report side effects early.

Counseling also plays a crucial role in setting realistic expectations, helping patients navigate the emotional and psychological impacts of their treatment. It facilitates informed decision-making, where patients understand the risks and benefits of their treatment options (Krist et al., 2017). Moreover, continuous patient education regarding lifestyle and dietary modifications can significantly improve their ability to manage side effects independently.

In conclusion, the management of side effects in patients receiving synthetic protease inhibitors for gynecological cancers requires a comprehensive and proactive approach.

Combining strategies for early detection and prevention, tailored pharmacological and non-pharmacological interventions, and robust patient education and counseling can significantly enhance patient outcomes. This holistic approach not only addresses the physical aspects of side effects but also supports the psychological and emotional well-being of patients, ultimately contributing to a more positive treatment experience and improved quality of life.

1.12 Future perspectives

The trajectory of synthetic protease inhibitors in the realm of gynecological cancers is an evolving narrative, marked by continuous research and innovation. As we stand on the cusp of new discoveries, the future promises more refined therapies with a focus on reducing side effects, enhancing safety profiles, and personalizing treatment approaches. This section explores these future perspectives, offering a glimpse into the potential advancements in this field.

Current research is intensely focused on deciphering the complex mechanisms underlying the side effects of synthetic protease inhibitors. This deeper understanding is guiding the development of next-generation inhibitors with enhanced specificity and reduced off-target effects. For instance, efforts are underway to design inhibitors that more precisely target cancer-specific proteases, thereby minimizing impact on healthy cells and tissues.

Additionally, research is exploring combination therapies where protease inhibitors are paired with other agents to synergize their effects while minimizing toxicities. The use of lower doses of each agent in these combinations can potentially reduce the severity of side effects without compromising therapeutic efficacy.

1.13 The potential role of personalized medicine in minimizing side effects

Personalized medicine stands at the forefront of future cancer therapy, offering a tailored approach to treatment based on individual patient characteristics (Krzyszczyk et al., 2018). This paradigm shift is particularly pertinent in the context of protease inhibitors, where genetic profiling can inform treatment choices and dosing strategies.

Genomic and proteomic analyses are enabling the identification of biomarkers that predict response to specific inhibitors and susceptibility to side effects (Tainsky, 2009). By integrating this genetic information, clinicians can select the most appropriate protease inhibitor for each patient, optimizing efficacy while minimizing the risk of adverse effects.

Furthermore, ongoing research into pharmacogenomics seeks to understand how genetic variations influence drug metabolism and toxicity (Vaidhya et al., 2024). This knowledge will allow for more precise dosing regimens, tailored to each patient's genetic makeup, thereby reducing the incidence and severity of side effects.

The biological basis of these side effects often relates to the inhibitors' mechanism of action. Since these drugs target enzymes also involved in normal physiological processes, their inhibition can disrupt essential bodily functions, leading to various side effects. Additionally, interactions with other medications can exacerbate these effects, while genetic predispositions and individual patient factors such as age, comorbidities, and previous treatment history can influence their severity and manifestation.

Managing the side effects of synthetic protease inhibitors involves a blend of pharmacological and nonpharmacological approaches. Pharmacological interventions may include medications to treat the side effects or mitigate their impact. Nonpharmacological strategies encompass lifestyle modifications, dietary changes, and supportive therapies such as physical therapy and psychological support.

An essential component of managing side effects is patient education and counseling. Informing patients about potential side effects, their symptoms, and when to seek medical help empowers them to be active participants in their care. Counseling aids in setting realistic expectations and helps patients navigate the emotional and psychological impacts of their treatment.

Looking ahead, the field of protease inhibitor therapy in gynecological cancers is ripe with potential. Ongoing research is focused on developing inhibitors with enhanced specificity and reduced off-target effects. Advances in drug design technologies are paving the way for the emergence of inhibitors with improved safety profiles. Personalized medicine, utilizing genetic profiling to inform treatment choices and dosing, stands at the forefront of future cancer therapy. This approach promises more effective, safer, and individualized treatment options, potentially transforming the landscape of gynecological cancer treatment.

1.14 Conclusion

In summary, synthetic protease inhibitors represent a significant advancement in the treatment of gynecological cancers. Their role in offering targeted and effective therapy options is undeniable. However, a comprehensive understanding and proactive management of the associated side effects are crucial to ensure patient safety and enhance treatment outcomes. The future of this therapy is promising, with ongoing research and innovation poised to further refine these treatments, enhancing their safety and efficacy. The journey of synthetic protease inhibitors in gynecological cancer treatment is a testament to the advances in medical science, emphasizing a patient-centric approach where the management of side effects is as crucial as the efficacy of the therapy itself.

Synthetic protease inhibitors, while heralding a new era in the treatment of gynecological cancers, come with a spectrum of side effects that necessitate careful consideration. Insights from preclinical study and clinical trials provide a roadmap for navigating these challenges, ensuring that the benefits of these potent agents are maximized while minimizing their adverse impacts on patients' lives. As research continues to advance, it is hoped that future developments will further refine these therapies, enhancing their safety and tolerability for patients battling gynecological cancers.

In conclusion, the future of synthetic protease inhibitors in gynecological cancers is one of great promise. With ongoing research aimed at understanding and reducing side effects, the emergence of new inhibitors with improved safety profiles, and the advent of personalized medicine, the next chapter in cancer therapy is poised to offer more effective, safer, and personalized treatment options. This progress not only represents a leap forward in cancer treatment but also embodies a more patient-centric approach, where the management of side effects is as crucial as the efficacy of the therapy itself.

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CHAPTER

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The role of synthetic protease inhibitors in breast cancer

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1. Breast cancer

Breast cancer is the most commonly diagnosed cancer worldwide, and it accounts for 25% of all prevalent cancers in females. Among women, it is the second most common cause of cancer-associated death. Its burden increased globally, with an estimate of 685,000 women dying in 2020. This accounts for 16% of cancer deaths in women (Arnold et al., 2022). The World Health Organization has launched a Global Breast Cancer Initiative with the aim of reducing the breast cancer burden through early diagnosis, timely treatment, and successful patient management. According to global statistics for 2020, a total of 685,000 women died of breast cancer. Across the globe, a wide variation has been observed in the incidence rates of breast cancer. In Asian and African countries, the incidence rate ranged from <40 per 100,000 females, while in other countries, including Northern America, parts of Europe, Australia, and New Zealand, it was 80 per 100,000 (Arnold et al., 2022). However, such wide geographical variations were not observed in mortality rates. Due to increasing population and aging, it has been estimated that globally, by the year 2040, breast cancer burden is most likely to increase by more than 3 million new cases and 1 million deaths per year (Arnold et al., 2022). In India, it is the second most common cancer, with a mortality rate of 25.8 per 100,000 women. However, in recent times, it has become the most common. The capital city of India (Arnold et al., 2022), New Delhi, was found to have the highest rate of age-related breast cancer, with 41 per 100,000 women (Malvia et al., 2017). Although breast carcinoma incidence is high in the postmenopausal group, it is currently found to occur more in Indian younger women.

Currently, enormous research is targeted to improve the clinical outcomes of breast cancer. Most of this research is mainly concentrated on improving breast cancer screening, diagnosis, therapeutic treatments, and clinical management. In breast cancer, three receptors, including

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estrogen, progesterone, and human epidermal growth factor-2, are expressed in the tumor cells. Breast cancer is divided into four types depending on the expression of these receptors. In type 1, the presence of the estrogen receptor and the complete absence of human epidermal growth factor-2, along with high-grade differentiation, are seen. In type 2, similar to type 1, the presence and absence of estrogen and human epidermal growth factor-2 are observed, along with intermediate to low-grade differentiation. In contrast, in type 3, there will be contentious expression of human epidermal growth factor-2 receptors, and in type 4, the absence of all these receptors is identified (Velesiotis et al., 2019). Among all types, type 4 is considered to be the most dangerous type due to the state of recurrence and high speed of metastasis.

The development and progression of breast cancer in its early stages are influenced by hormones. Cytochrome P450-associated metabolic pathways, prolonged exposure to continuous exposure to hormonal receptors, and the induction of hormone-mediated aneuploidy are implicated in breast cancer carcinogenesis (Kulendran et al., 2009). Currently, chemotherapy is most commonly employed to treat breast cancer. Among the various drugs used in chemotherapy, abitrexate, carboplatin, and abraxane are popular ones in breast cancer treatment (Bakrania et al., 2017). It is noteworthy to mention that chemotherapy is associated with side effects. Hence, there is a need to explore alternative treatments or therapies that are targeted for treatment. The specific genes and proteins involved in cancer cell growth and survival are the options to target such an alternative treatment. Potent targeted enzyme inhibitors with minimal or no side effects are exclusively studied for the treatment of various cancers, including breast cancer (Ma et al., 2020).

2. Proteases

Proteases are destructive enzymes that hydrolyze the peptide or amide bonds of a protein into polypeptides or individual amino acids. They are recognized at the very beginning of protein evolution and are responsible for maintaining protein homeostasis in primitive organisms. They embark on various diverse roles in biosystems by interfering in the biochemical, physiological, and regulatory functions of organisms. Protases constitute around 6% of human genome proteins and also account for 1%–5% of the genomes of other organisms such as bacteria and viruses (Barrett, 2004). Structurally, they ranged from single-domain catalytic molecules to multifunctional macromolecules. The amino acid core in the active site differs among various proteases. Proteases are classified into six categories, including cysteine, serine, threonine, glutamic acid, aspartate proteases, and metalloproteases, depending on the amino acid core in the active site as well as the catalytic mechanism of peptide bond cleavage (Eatemadi et al., 2017; Tyndall et al., 2005).

These main categories of proteases are further subclassified based on their specificity and/or protein fold. In the protease classes of cysteine, serine, and threonine, enzymatic hydrolysis includes the formation of an acyl enzyme complex and the subsequent release of carboxylate and amine end products. On the other hand, aspartic, glutamic, and metalloprotease classes catalyze the substrate peptide bonds by using a nucleophile with an activated water molecule. Barrett et al. (2012) and Leung et al. (2000) explained the mechanisms of action of these

protease classes. Proteases are necessary for living organisms; therefore, they are freely available in many sources of biological systems, including plants, animals, and microorganisms such as viruses, fungi, and bacteria. The characteristic properties of proteases are ubiquitous: temperature, pH, catalyst requirement, mechanism of action, and stability. These characteristics enable them to be used in various commercial applications in addition to providing health benefits (Raghunath et al., 2010). These applications include various industries such as pharmaceuticals, detergents, leather, dairy, food, brewing, soy sauce production, meat tenderization, etc.

Proteases are involved in the regulation of various proteins synthesis, turnover, and function. Through these activities, they control biological processes including protein degradation, embryogenesis, immune responses, wound healing, cell proliferation, differentiation, and migration, as well as apoptosis. Any changes in their expression and subsequent enhanced activity are linked to the propagation of many diseases, including cancer, neurodegenerative diseases, cardiovascular, respiratory, and inflammatory diseases. They are also associated with infectious diseases caused by fungi, parasites, viruses, and bacteria. Hence, antagonization of proteases has been successfully involved in designing promising therapeutic drugs against these diseases (Rudzińska et al., 2021). The dysfunction of proteases is well known to be associated with various pathological conditions in both humans and animals. Henceforth, these enzymes have been used as attractive targets for the development of new drugs for many diseases (Abbenante and Fairlie, 2005).

3. Protease inhibitors

PIs are naturally found in various leguminous plants and cereals. In plants, their biological role is to protect them from insects as well as herbivores. The basic fact of the irreversible activity of proteases on peptide bonds enabled the evolution of extensive protease inhibitors with an extensive regulatory network for controlling protease activity. PI are classified into two large groups depending on the structural dichotomy: (1) low-molecular-weight peptidomimetic inhibitors and (2) protein protease inhibitors with peptide chains. Further, they were also classified into five classes based on their inhibitory activity at the protease active site. These classes include aspartyl, serine, threonine, metalloprotease, and cysteine inhibitors (Fear et al., 2007). It's worthwhile to note that few PIs have the ability to inhibit more than one protease. For instance, serine family−related PIs are active against both serine and cysteine proteases (Fear et al., 2007).

PI can act through the following mechanisms while inhibiting proteolytic hydrolysis: one is through irreversible trapping reactions, and the other is through reversible tight-binding reactions (Rawlings et al., 2004). In the former mechanism, PI alters the conformation by trapping the enzyme molecule covalently and cleaving an internal peptide bond. However, both inhibitors and proteases do not involve further reactions. In the second mechanism, PI attaches directly to the protease active site, and these interactions are reversible. After reversing the enzyme, the inhibitor can be either in an actual state or a modified state. Conversely, in both mechanisms, information regarding the structure of proteases and their active sites is crucial when introducing specific inhibitor molecules. Any living organism utilizes the PIs

to prevent self-damage by regulating the proteolytic activity of proteases (Neurath, 1999). PIs are also used for self-defense against pathogens as well as predators (Xue, 2019; Sabotič et al., 2012). Currently on the market, there are many commercially available PIs for treating various human diseases. Dabigatran PI is used for the treatment of pulmonary embolism and angiotensin-converting enzyme inhibitors for hypertension (Di Nisio et al., 2005; Agbowuro et al., 2017). Another PI, bortezomib, is used for the management of multiple myeloma as well as for fungal infections (Merin and Kelly, 2015). PI has been studied extensively against hypertension and human immunodeficiency virus (HIV). PI is used either along with or along with reverse transcriptase inhibitors to decrease the viral load in HIV patients (Arribas et al., 2005). PI has been reported to treat advanced stages of clinical development and their corresponding therapeutic targets.

In addition to the regulation of imperative biological processes, including gene expression, cell proliferation, differentiation, and apoptosis, it also participates in carcinogenesis at primary as well as metastatic sites (Egeblad and Werb, 2002). Now it is clear that tumor cells promote the expression of proteolytic enzymes in nearby nonneoplastic cells, affecting their activity to promote tumor expansion (Zucker et al., 2000). On the other hand, proteases influence tumor progression by improving the nutrient and oxygen supply to the tumor cells from surrounding tissues and organs (Yang et al., 2009). Evidence suggests that increased protease levels are found at the site of early stages of tumors and help in their proliferation, inflammatory cell recruitment, invasion, and angiogenesis (Eatemadi et al., 2017).

4. Protease inhibitors in cancer

PIs, high-molecular-weight proteins, are identified for their potential therapy against cancer progression. As proteases are involved in most of the steps of the cancer pathogenesis process, protease inhibitors have been noted for their role in cancer therapy. In humans, epidemiological studies have reported the anticarcinogenic properties of these compounds. Both natural and synthetic PIs are well known to be involved in the prevention of chemical and radiation-associated carcinogenesis.

Designing the PIs for cancer therapy is highly complicated due to the involvement of different proteases at different stages of cancer development. Additionally, all classes of proteases can be inhibited by a single PI. Troll & Kennedy, for the first time, identified the PI as preventing tumor promotion (Troll and Kennedy, 1989). In the rats, the PI "leupeptin" in the diet inhibited tumorigenesis in the colon, esophagus, and mammary glands (Troll et al., 1986). Later, synthetic PIs were found to inhibit skin carcinogenesis (Troll et al., 1970). The experimental and epidemiological evidence found that preventing or lowering protein digestion by PIs is the main mechanism behind their positive effects (Troll et al., 1984). PIs are generally thought to affect the enzyme system, which is associated with the regulation of transformed phenotypes. For example, chymotrypsin inhibitors are involved in the suppression of tumor growth by locking the digestive enzymes and indirectly thereby stopping the nutritional requirement of growing tumor cells (Troll et al., 1984). Another classic example of this mechanism is that some of the PIs suppress carcinogenesis by inhibiting the cellular enzymes that are involved in the transformation. Chymotrypsin inhibitors and Bowman-Birk

inhibitors prevented the malignant transformation by blocking the enzymatic cleavage of a peptide substrate (Billings et al., 1991). PIs are known to be involved in the prevention of cancer by interfering with cell invasiveness, the processing of growth factors at the genetic level, and the oxygen cascade (Das and Mukhopadhyay, 1994). The anticarcinogenesis effects of PIs are also associated with suppression of platelet aggregation and collagenase activation, influencing oncogene expression, and altering the immune response (Das and Mukhopadhyay, 1994).

The protease activity is inhibited by both exogenous and endogenous inhibitors. Among the endogenous inhibitors, tissue inhibitors of metalloproteinases (TIMPs), serpins, and cystatins are the key regulators. The majority of PIs can act on more than one type of protease and interact with more than one type of protease. For example, serpins interact with both serine protease and cysteine proteases (Rudzińska et al., 2021).

Cystatin is the endogenous cysteine PI that controls cathepsins in both tissues and cells. The cystatin superfamily inhibits various cysteine peptidases, including cathepsins B, H, and L (Folgueras et al., 2004). Cystatins prevent carcinogenesis by controlling tumor growth, invasion, and metastasis. In experimental studies, cystatins have been shown to suppress the metastasis or invasion of various cancers by limiting the lysosomal or inflammatory-related extra cysteine protease activity (Cox, 2009). Irreversible specific CtsB and CtsL are irreversible cathepsin inhibitors developed from epoxysuccinate derivatives that have been shown to be effective against breast cancer metastasis (Katunuma, 2011). On the other hand, CtsK, the reversible inhibitor, was effective against the tumor-induced disruption of cortical bone as well as osteolytic lesions (Duong et al., 2014). It also reduced the skeletal tumor burden in an experimental model of breast cancer bone metastasis (Duong et al., 2014; Duong et al., 2014).

Matriptase, a type of serine protease involved in a few epithelial cancers, was found to be inhibited by hepatocyte growth factor activator inhibitor-1. This inhibitor has the potential for chemopreventive interventions in human prostate cancer (CaP) (Saleem et al., 2006). Serpins are the other group of serine PIs implicated in various cancer types, including breast cancer, ovarian cancer, head and neck cancer, prostate cancer, lung cancer, cervical and endometrial cancer, etc. Proteasome inhibitor (PSI) inhibits the chymotrypsin-like activity of 20S threonine protease (Oyajobi and Mundy, 2003). PSI reduced the tumor accumulation of plasmacytoma cells in mice and also inhibited osteolytic lesions growth (Oyajobi and Mundy, 2003).

Matrix metalloproteinases (MMPs) are zinc-dependent nonserine proteases associated with invasive and metastatic cancer. They involve the breakdown of various structural proteins and promote hematogenous metastasis. MMP inhibitors consist of TIMPs and synthetic agents. Current evidence suggests that MMP activity is elevated in breast cancer. Especially MMP-2 and MMP-9 were found to be involved in all stages of tumor growth (Radenkovic et al., 2017). Marimastat, one of the MMP inhibitors, was tested in various phase III clinical trials (Rasmussen and McCann, 1997) and in an advanced pancreatic cancer trial (Bramhall et al., 2001). Marimastat at a higher dose was found to be effective, similar to conventional therapy. This inhibitor was also observed to be effective in gastroesophageal adenocarcinoma patients (Fenteany et al., 1995).

5. Protease inhibitors in breast cancer

Proteases play a significant role in pathological circumstances and physiological processes. including the immune response to breast cancer. The progression of breast cancer depends on the activation as well as the level of the protease. Serpins and cathepsins, particularly cathepsin D (CatD) and MMPs, are known to be involved in processes of invasion and metastasis of breast cancer. The overexpression of the classical extracellular protease mesotrypsin, also known as serine endopeptidase, was found in the tumor microenvironment of breast cancer. In an in vitro study on the T4-2 breast cancer cell line, this protease was upregulated, whereas suppressing this protease reduced the malignant cells (Hockla et al., 2010). Extensive evaluation of the molecular pathways of breast cancer will definitely help to identify and/or design protease-based therapeutic targets.

6. Synthetic compounds with protease inhibitory activity in breast cancer

6.1 Marimastat

MMPs are enzymes vital for tissue remodeling and normal physiological processes such as wound healing. Elevated MMP activity is noted in various conditions, including cancer, where MMPs help overcome barriers to tumor growth (Rasmussen and McCann, 1997). Marimastat, an inhibitor targeting major MMPs, holds potential as an anticancer agent by impeding the spread and growth of tumors in animal models. Marimastat is a synthetic inhibitor of MMPs and is considered a second generation of anticancer drugs (Xia et al., 2022; Steward, 1999). Marimastat, a low-molecular-weight peptide mimetic, incorporates a hydroxamate group that binds to the zinc atom within the active site of MMPs. This compound effectively inhibits a wide range of MMPs and demonstrates activity in various human tumor xenograft models (Thomas and Steward, 2000). A preliminary trial by Miller KD. et al. assessed marimastat's tolerability in early-stage breast cancer patients, revealing manageable side effects and suboptimal plasma levels. While effective in phase II trials, phase III results in advanced malignancies varied, highlighting the challenges in predicting their impact on survival (Miller et al., 2002). The use of marimastat following first-line chemotherapy for metastatic breast cancer does not extend progression-free survival (Sparano et al., 2004). Moreover, patients with elevated levels of marimastat experienced musculoskeletal toxicity, and this toxicity was linked to poorer overall survival (Sparano et al., 2004). The analysis of plasma MMP-7 or MMP-9 levels, as performed in the study by Zucker et al., did not provide significant prognostic or predictive information for individuals with metastatic breast cancer or those undergoing marimastat treatment (Zucker et al., 2006).

6.2 Batimastat

Batimastat, also identified as BB-94, functions as a metalloproteinase activity inhibitor by binding to the zinc ion within the active site of MMPs (Low et al., 1996). In a nude mouse xenograft model, batimastat demonstrates inhibition of regrowth and metastasis in human breast cancer. The potential mechanisms underlying batimastat's inhibitory activity exclude direct cell toxicity or alterations in MMP or TIMP mRNA levels (Sledge et al., 1995). While batimastat significantly

slows tumor growth without causing regression or inhibiting local invasion, it triggers an increase in murine urokinase plasminogen activator (uPA) expression at the tumor-stromal junction, suggesting a response to MMP inhibition (Holst-Hansen et al., 2001). In uPA-deficient mice, tumor growth is slowed, and additional batimastat treatment does not further inhibit growth. These findings underscore the intricate nature of tumor progression and carry implications for the clinical application of MMP inhibitors (Holst-Hansen et al., 2001). Moreover, analysis of images from treated tumors showed that batimastat inhibited the regrowth of both tumor and stromal components, a process typically observed following short-term IL-12 therapy (Scott et al., 2000). The metalloproteinase inhibitor BB-94 effectively inhibited human epidermal growth factor receptor and Erk activation in resistant cells. Targeting both HER3 and EGFR was deemed crucial for addressing fulvestrant-resistant cell growth (Kirkegaard et al., 2014). In a study by Low J. A. et al., the hormone-independent MDA435/LCC6 human breast cancer cell line was used to create solid tumors in athymic nude mice. Treatment with 50 mg/kg batimastat significantly decreased tumor size. However, when the same cell line was used to induce malignant ascites in mice, batimastat did not increase survival or suppress ascites formation, contrary to previous literature. Nevertheless, treated animals exhibited notable tumor cell consolidation and reduced dispersion of ascites cells compared with controls (Low et al., 1996).

6.3 Prinomastat

Prinomastat is a powerful and selective oral inhibitor that targets matrix metalloproteinase-2, -9, -13, and -14. It has demonstrated effectiveness in suppressing tumor growth and angiogenesis across various preclinical models, such as colon, breast, lung, melanoma, and glioma. Additionally, the combination of prinomastat with certain chemotherapeutic agents has displayed additive effects, indicating its potential in enhancing the therapeutic impact (Scatena, 2000). Marlene Wiart et al. demonstrated that magnetic resonance imaging (MRI) effectively detects early alterations in microvascular permeability in xenograft tumors following treatment with the MMP inhibitor prinomastat. Administered twice daily for 1.5 days, prinomastat swiftly reduced microvascular permeabilities ($P < .05$), with the effect disappearing by the 10th day of treatment. This study highlights the potential of contrast-enhanced MRI as a biomarker for tracking dynamic MMP effects in cancers (Wiart et al., 2004). In another study, Elena I Deryugina et al. investigated the impact of prinomastat, an MMP inhibitor, on breast carcinoma cell migration. The focus was on membrane type 1 matrix metalloproteinase (MT1-MMP) and alphavbeta3 integrin, crucial for tumor cell dissemination. In MCF7 cells expressing both, MT1-MMP processed pro-alphav integrin, promoting alphavbeta3 integrin maturation and cell migration on vitronectin. Prinomastat, while blocking MT1-MMP-mediated matrix degradation, increased migration of MCF7 cells by inhibiting MT1-MMP-dependent vitronectin proteolysis. The findings suggest MT1-MMP may enhance cell migration by modifying cell surface receptors, such as alphavbeta3 integrin (Deryugina et al., 2003).

6.4 Viracept (nelfinavir)

Nelfinavir (NFV), initially recognized for its anti-HIV properties, demonstrates antiproliferative effects in breast cancer cells. NFV exhibits a range of anticancer effects. Its extensive

activity, oral accessibility, and recognized administration make it a promising candidate for repurposing as a cancer therapeutic, especially since it is already approved by the FDA (Gills et al., 2007). While the precise molecular mechanism remains uncertain, it is linked to the inhibition of the Akt pathway (Liebscher et al., 2017). Soprano M et al. found that in experiments involving both breast cancer and normal cells, NFV selectively reduced the viability of cancer cells through apoptosis and necrosis without affecting normal cells (Soprano et al., 2016). The drug altered the cellular redox state, inducing intracellular ROS production specifically in cancer cells. NFV disrupted the Akt-HSP90 complex, resulting in Akt degradation, and this effect was dependent on ROS. The study underscores the enhancement of ROS as the primary mechanism driving NFV's anticancer activity in breast cancer cells, supporting its potential in developing novel oxidative molecules for breast cancer therapy (Soprano et al., 2016). Shim JS et al. conducted a study aiming to identify new drug targets for aggressive HER2-positive breast cancer. Through pharmacological profiling, they discovered that the anti-HIV drug NFV selectively inhibits HER2-positive cells by targeting heat shock protein 90 (HSP90). In vivo experiments demonstrated NFV's efficacy in selectively inhibiting HER2-positive breast cancer growth and overcoming resistance in trastuzumab- and/or lapatinib-resistant cells. This finding positions NFV as a novel HSP90 inhibitor, suggesting its potential for HER2-positive breast cancer treatment trials with an established HIV patient dosage regimen (Shim et al., 2012). Another study by Brüning A et al. revealed the ability of NFV to induce apoptosis across various cell lines. Moreover, combining of NFV with tamoxifen, an established breast cancer treatment, proved synergistic, reducing the concentration required for effective apoptosis induction. Tamoxifen further enhanced endoplasmic reticulum stress and autophagy, irrespective of estrogen receptor status, suggesting potential efficacy for hormone-nonresponsive tumors. The study advocates for clinical trials to assess the therapeutic effectiveness, safety, and tolerability of this promising NFV-tamoxifen combination in breast cancer treatment (Brüning et al., 2010). A study by Chakravarty G et al. revealed that MCF-7/doxorubicin (DOX) cells exhibited 15–20-fold higher resistance to DOX-induced cytotoxicity, which NFV significantly reduced. Multiple NFV exposures reduced DOX resistance, inhibiting P-glycoprotein expression and promoting intracellular DOX accumulation. NFV also suppressed cell-survival markers (AKT) and autophagy, induced unfolded protein response, and triggered endoplasmic reticulum stress-induced death sensors. In a mouse xenograft model, combined NFV and DOX treatment effectively reduced tumor growth, suggesting NFV as a promising adjunct to chemotherapy for overcoming multidrug resistance in breast cancer cells (Chakravarty et al., 2016). It has also been reported that NFV effectively inhibits the activity of breast cancer resistance protein (BCRP) in human embryonic kidney cells expressing wild-type BCRP. Although NFV shows inhibitory effects, it is not actively transported by BCRP. This suggests that NFV can function as an inhibitor but is not a substrate for BCRP. The study emphasizes the potential significance of BCRP in drug–drug interactions, particularly when coadministering NFV with drugs that are substrates of this transporter (Gupta et al., 2004).

6.5 Ritonavir

Ritonavir, mainly prescribed as a protease inhibitor for HIV/AIDS, is currently being investigated for potential applications in treating certain types of cancer (Talha et al., 2024). In the study conducted by Gote V et al., the investigation centered on ritonavir's

potential to overcome multidrug resistance (MDR) and treat triple-negative breast cancer (TNBC). They designed nanomicelles with hyaluronic acid (HA) to encapsulate paclitaxel (PTX) and ritonavir (RTV). Targeting CD44 receptors in breast cancer, these nanomicelles effectively entered and induced cytotoxic effects in metastatic breast cancer (MBC) and TNBC cell lines. Additionally, they successfully reversed MDR by inhibiting P-glycoprotein, suggesting promise for addressing both MDR and TNBC (Gote et al., 2021). In a mouse model of breast cancer that mimics the natural environment and retains immune competence, ritonavir alone did not exhibit a direct antitumor effect. However, when ritonavir was combined with docetaxel treatment, an enhanced efficacy was observed in inhibiting tumor growth (Hendrikx et al., 2016). Ritonavir inhibits breast cancer growth by targeting Hsp90 substrates, including Akt. Its potential in breast cancer therapeutics is significant, and its efficacy may be enhanced through prolonged exposure or by interfering with Hsp90 RNA (Srirangam et al., 2006). Ritonavir was found to be effective in reversing resistance in cells overexpressing MRP9, exhibiting a resistance factor of 1.4 (Bierman et al., 2010).

6.6 γ-Secretase inhibitors

The γ-secretase complex serves as a vital hydrolase for multiple type 1 transmembrane proteins, playing a pivotal role in activating the Notch receptor and overseeing the transcription of target genes (Han and Shen, 2012). Irregular activation and expression of the Notch pathway are strongly linked to the initiation and advancement of various types of tumors, including breast cancer (Jia et al., 2021; Peltonen et al., 2013). Over the past two decades, the targeting of γ-secretase, a crucial regulator in the Notch pathway, has led to the development of numerous γ-secretase inhibitors (GSIs) undergoing clinical investigation (Nie et al., 2021). Originally designed for Alzheimer's treatment, GSIs have found new purpose as anticancer agents due to their ability to impede the cleavage of the Notch receptor (McCaw et al., 2021). In a phase 1b dose escalation trial, Means-Powell et al. explored the combination of GSI RO4929097 with exemestane in patients with ERα+, metastatic breast cancer. The study suggests that further investigation is warranted for combinations involving endocrine therapy and Notch inhibitors, particularly in the context of endocrine-resistant ERα-positive breast cancer (Means-Powell et al., 2022). Utilizing breast cancer cell line xenografts and patient-derived xenografts (PDX), Wang D et al. illustrated that the simultaneous administration of GSI (MK-0752) and tocilizumab not only decreases breast cancer stem cells but also impedes tumor growth. This combination presents a promising and innovative therapeutic strategy for addressing Notch3-expressing breast cancer in women (Wang et al., 2018). Another study focused on creating solid lipid nanoparticles conjugated with death receptor-5 (DR5) antibodies (DR5-DAPT-SLNs) was to enhance the delivery of the ++γ-secretase inhibitor DAPT into cancer cells. Findings revealed that the improved cytotoxicity of DAPT is attributed to DR5-mediated uptake. An in vivo anticancer experiments demonstrated that DR5-DAPT-SLNs induce more significant tumor regression compared with both DAPT-SLNs and the bulk drug. The study emphasizes the selective targeting of cancer cells by DR5-DAPT-SLNs, leading to enhanced anticancer efficacy of DAPT, especially against triple-negative breast cancer (TNBC) cells (Pindiprolu et al., 2021). NMK-T-057, a compound binding to the γ-secretase complex, demonstrated inhibitory effects on breast

cancer cell proliferation, colony formation, and motility, with minimal cytotoxicity to noncancerous cells. It induced significant autophagic responses, leading to apoptosis. In a 4T1-BALB/c mouse model, NMK-T-057 inhibited tumor progression. This suggests its potential as a breast cancer drug candidate, triggering autophagy-mediated cell death by inhibiting γ-secretase-mediated Notch signaling activation (Das et al., 2019). The GSI MRK-003, a Notch signaling antagonist, effectively hindered tumor sphere-derived cell survival and eradicated tumor-initiating cells (TICs) in vitro and in mice. It reversibly restrained mammosphere-resident cell self-renewal without compromising survival. In tumor-bearing mice, MRK-003 administration led to rapid, enduring tumor regression by eliminating TICs and inducing apoptosis and differentiation. This underscores MRK-003's potential for ensuring long-term, recurrence-free survival by targeting breast TICs within tumors (Kondratyev et al., 2012). Treating breast cancer transplants on chick embryo chorioallantoic membranes with nanoparticles efficiently reduced the cancer stem cell population, suggesting that designing nanoparticles based on specific cancer stem cell characteristics can enhance targeted drug delivery and therapy for cancer stem cells (Mamaeva et al., 2016). Z-LLNle-CHO, a GSI I, exhibits toxicity against breast cancer cells in both in vitro and in vivo settings. Notably, this cytotoxic effect in breast cancer cells is attributed to proteasome inhibition rather than γ-secretase inhibition (Han et al., 2009).

Sulindac sulfide (SS), a nonimmune suppressive gamma-secretase modulator, has been confirmed as a Notch1 cleavage inhibitor in triple-negative breast cancer (TNBC) cells, exhibiting notable antitumor efficacy in TNBC models. SS, without impacting T cell Notch cleavage, demonstrated impressive results in a mouse TNBC tumor model (C0321). Combining SS with α-PD1 immunotherapy enhanced its antitumor effects in TNBC organoids and in vivo. This suggests potential repurposing of the FDA-approved, safe SS for TNBC treatment, either alone or with chemotherapy/chemo-immunotherapy, offering a cost-effective and swiftly applicable therapeutic option for TNBC patients needing more effective treatments (Hossain et al., 2023). Zhang CC et al. investigated the synergistic impact of the γ-secretase inhibitor PF-03084014 in combination with docetaxel in breast cancer models. Their study revealed synergistic effects between PF-03084014 and docetaxel through multiple mechanisms, suggesting a robust preclinical basis for considering the clinical application of PF-03084014 to enhance taxane therapy (Zhang et al., 2013). In another phase I clinical trial, PF-03084014, a discerning gamma-secretase inhibitor, was examined in patients with advanced triple-negative breast cancer (TNBC). The coadministration of PF-03084014 with docetaxel in individuals with locally advanced or metastatic TNBC exhibited a well-tolerated safety profile and presented modest but encouraging initial antitumor activity (Locatelli et al., 2017). Combining LY411575, a GSI, with suberoylanilide hydroxamic acid enhances treatment efficacy by blocking the Notch pathway. This cotreatment promotes apoptosis, generates reactive oxygen species, disrupts mitochondria, reduces stemness properties, induces autophagy-independent cell death, and decreases expression of inflammatory cytokines, along with downregulating Notch downstream genes and mesenchymal markers (Sen and Ghosh, 2023). In an ERBB2 transgenic mice breast cancer model, the inhibition of the Notch pathway by a gamma-secretase inhibitor results in the attenuation of AKT/mammalian target of rapamycin (mTOR) signaling and a decrease in glucose uptake (Efferson et al., 2010). In addition, GSI was found to enhance the sensitivity of MDA-MB-231 cells to doxorubicin. The combination of GSI with doxorubicin inhibited cell proliferation and induced

apoptosis. This effect was associated with changes in key markets, including increased expression of PTEN and Bax, and decreased expression of Notch-1, HES-1, CyclinD1, and Bcl-2 (Li et al., 2015).

6.7 Aprotinin

In 1930, the discovery of aprotinin marked its recognition as a powerful pan-protease inhibitor. Its antiviral properties, particularly in preventing and treating acute respiratory virus infections, stem from its ability to inhibit the proteolytic activation of certain viruses (Ivachtchenko et al., 2023). Aprotinin, functioning as a nonspecific serine protease inhibitor, also demonstrates efficacy in inhibiting the plasminogen−plasmin system and matrix metalloproteinases (Soleyman-Jahi et al., 2019). Aprotinin exerted a notable growth-inhibitory effect on various cell lines, including MDA-MB-231, SK-BR-3, and MCF-7 in human breast cancer, as well as the normal fibroblast cell line HDF-1. The inhibitory impact on cell growth was particularly pronounced in cancer cell lines. Additionally, aprotinin exhibited significant suppression of local invasion, particularly evident in the case of MDA-MB-231 (Soleyman-Jahi et al., 2019).

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CHAPTER

35

Cathepsin-responsive nanomaterials for targeted delivery of malignant tumors

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1. Introduction

According to the World Health Organization, cancer is one of the life-threatening diseases worldwide, and the morbidity of the disease is rising daily. Early diagnosis and treatment are vital for health management (https://www.who.int/news-room/fact-sheets/detail/cancer). Currently, conventional chemotherapy is the chief treatment for highly proliferating cancer cells and has limitations such as poor solubility of drugs, weak bioavailability, fast blood clearance, and unavoidable tissue toxicity (Cho et al., 2008). Broad research on tumor biology recently resulted in the development of potential therapeutic targets (Parodi et al., 2022; Soltani et al., 2022; Soond et al., 2020). Many novel drug delivery systems (DDSs) are currently available to direct bioactive components to tumor sites. Hence, it is essential to develop tumor-specific, stimuli-responsive DDSs for improving the therapeutic efficacy and reducing toxicity to cancer (Gu et al., 2018; Mura et al., 2013). Scientists have designed novel DDSs with nanomedicine for directing bioactive components to the tumor sites to resolve this disease condition. According to recent research methodologies, nanoparticles are addressed as the new generation of diagnostic and therapeutic tools. The nanoparticles' physicochemical

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properties, such as composition and size, make them highly tunable for varied purposes. The therapeutic studies conducted with nanoparticles have been intended for delivering different antitumorigenic substances into the sites of the tumor environment. The anticancer drugs used for the studies are mostly encapsulated/incorporated into different nanoparticles of choice, such as polymeric nanoparticles, lipid nanoparticles, protein nanoparticles, organometallic nanoparticles, or viruses-like nanoparticles, in which the cancer cells are endocytosed. Many researchers have assessed that for passive diffusion of nanoparticles into the sites of the tumor, the size of the nanoparticles is one of the significant factors, and for the effectual uptake, the size of the nanoparticles must be less than 200 nm (Yuan et al., 1995; Hobbs et al., 1998; Peer et al., 2007). Thus, nanomedicine has opened a new stage of cancer treatment, and the combination of nanotechnology and medicine deserves more in-depth research. This chapter provides some aspects on the importance of different cathepsin-responsive nanocarrier systems in treating malignant tumors and in imaging studies.

2. Drug delivery systems for cancer therapy

In nanomedicine, DDSs are crucial in answering many questions about delivering therapeutic agents to the tumor sites. Over the last few decades, broad research interest from academia and industry has been going on to design nanoscale systems for anticancer drug delivery. For that the nanoscale systems might be non-immunogenic, biocompatible, and biodegradable. In the past decades, various innovative drug delivery methods are being used for the treatment of cancer, which includes materials like polymers, lipids, proteins, scaffolds, and virus-like particles for developing therapeutics in cancer treatment (Lee et al., 2016; Kim et al., 2023). In cancer research, nanocarriers are classified into two types based on their targeted and non-targeted delivery of drugs. This includes both organic and inorganic nanocarriers.

2.1 Organic nanocarriers

Organic nanocarriers use organic molecules as core for drug encapsulation. This group comprises polymeric nanoparticles, considered one of the modest materials for drug delivery, and these are biocompatible, colloidal, and biodegradable with high drug encapsulation efficiency, biodistribution, and pharmacokinetic control (Park et al., 2008; Parveen and Sahoo, 2008). The polymeric nanoparticles can be synthetic and natural based on drug delivery applications. Due to the sustained drug release, synthetic polymers have more advantages than natural polymeric nanocarriers (Senapati et al., 2018). Another type of organic carrier is liposomes, which are self-closed spherical structures with concentric lipid bilayers in the middle with an encapsulated aqueous phase and have an inimitable capacity to incorporate many hydrophilic drugs, DNA, RNA, etc. The drug is mainly encapsulated in the inside core of liposomes which is aqueous, and incorporated within lamellae when it is a hydrophobic drug, which makes liposomes as functional therapeutic carriers in drug delivery. Protein-based nanocarriers deal with several uses, such as binding capacity, nontoxic nature, biocompatibility, and long half-life in circulating plasma, e.g., albumin. Studies reported that human

serum albumin nanoparticles loaded with anticancer drug doxorubicin (Dox) have greater antitumor capacity than the drug alone without nanoparticles against UKF-NB3 and IMR 32 cell lines (Dreis et al., 2007). While hydrogels are polymeric and hydrophilic, three-dimensional (3D) networks have a sizable absorbing capacity of water or biological fluids. They are cytotoxic as well as biocompatible and can act as a delivery agent of drugs/therapeutics in normal cell line (COS7) and cancer cell lines (HepG2 and A549) (Senapati et al., 2018).

Virus-like nanoparticles (VLPs) are future scope as nanocarriers, which are polymer-based nanoparticles, can imitate the virus' structure through the ordered assembly of viral coat proteins. They lack viral genomes and have a uniform structure and controllable assembly. Recently, VLPs have paid vast consideration to nanotechnology-based medical diagnostics and therapies. This includes therapy, imaging, and theranostics in cancer research, as these can encapsulate or incorporate various agents involved in therapeutic and diagnostic purposes. Like other organic carriers, these are also biocompatible and biodegradable, which can be modified chemically or genetically (Kim et al., 2023). There are studies reported that using VLPs of bacteriophage P22, peptide drugs can be delivered intracellularly. These peptides are loaded covalently and have been reported with synergistic cytotoxicity and will release as it is stimulated by cathepsin B. These VLPs competently deliver peptides against MDA-MB-231 tumor cells and are reported to have applications in multifunctional theranostics (Wang et al., 2018). In addition, there are reports that human immunodeficiency virus (HIV) VLPs are able to deliver Cas9 cargo proteins N-terminally fused to an HIV protease site on the C-terminus of a Gag protein to localize the Cas9 into the VLP interior. The loaded HIV VLPs successfully transport Cas9 to edit the genomes of human T cells effectively without the need for electroporation.

2.1.1 Inorganic nanocarriers

Inorganic nanocarriers usually have metals as a core and are widely used for therapeutic and imaging purposes for cancer treatment due to their broad surface area, increased drug encapsulating capacity, low side effects, increased bioavailability, and controlled release of drug (Senapati et al., 2018). In the treatment of cancer, inorganic nanoparticles such as mesoporous silica-based nanoparticles, layered double hydroxides, quantum dots (QDs), magnetic nanoparticles, and carbon nanotubes were studied. Gao et al. (2004), in their study, reported the synthesis of bioconjugated QD probes with polymer encapsulation for targeting malignant tumor cells and reported the imaging studies by in vivo methods. For imaging and targeted studies on cancer cells, D-α-Tocopheryl polyethylene glycol 1000 succinate monoester (TPGS)-coated multifunctional (theranostic) liposomes have been developed in the form of docetaxel and QD by some groups (Muthu et al., 2012). Moreover, for diagnosis and image-guided chemotherapy, multifunctional QDs has also been reported as a targeted drug delivery agent in many cancer studies (Huang et al., 2015; Li et al., 2015). Carbon nanotubes (CNTs), another inorganic nanocarrier widely used in tumor therapy, mainly these are used in near-infrared photothermal ablation therapy in cancer research. In this targeted therapeutic approach, the surface functional groups of CNT or through CNT polymer coatings, the drug molecules are conjugated usually via cleavable bonds (Huang et al., 2010). Fullerenes are another class of inorganic carriers that are extensively used in cancer therapy. These are allotropes of carbon that perform like electron-deficient alkenes and respond readily with

electron-rich species (Yadav and Kumar, 2008). Likewise, mesoporous silica nanoparticles (MSNs), calcium phosphate nanoparticles, and superparamagnetic iron oxide nanoparticles (SPIONs) are widely used as drug-delivery vehicles in different cancer studies (Lai et al., 2003; Lee et al., 2014; Widder et al., 1980).

3. Cathepsin family

Cathepsins are lysosomal proteases in acidic endo/lysosomal compartments, which mainly function in the degradation of protein/peptides and are widely distributed in tissues (Klein et al., 2018). They are produced as proenzymes and become active by processing. These proteins are considered significant signaling molecules playing diverse crucial roles. Depending on their active site, amino acid, structure, catalytic mechanism, and substrate, cathepsins are divided into A, B, C, D, E, F, G, H, L, K, O, S, V, and W (Overall and Blobel, 2007; Reiser et al., 2010; Turk et al., 2012). Cathepsins D and E are aspartic proteases, cathepsins B, C, F, H, K, L1, L2/V, O, S, W, X/Z) are cysteine proteases, whereas cathepsins A and G are serine proteases. Different types of cathepsins with their functions are represented in Table 35.1.

In normal condition, from the endoplasmic reticulum (ER), cathepsins are synthesized as preprocathepsins. An N-terminal signal peptide leads the preprocathepsins into the ER lumen; there, it is cleaved to procathepsins by a signal peptidase. In the ER, N-linked glycosylation occurs, leading to increases mannose level in procathepsins. When procathepsins reach the Golgi complex, the mannose residues were modified to mannose 6-phosphate (M6P). M6P-procathepsins are bound to M6P receptors in the Golgi complex and sorted directly either to endolysosomes or indirectly to the plasma membrane, and it will enter into endolysosomes. Once it enters into the endolysosomes, the M6P receptors are transported back to Golgi network. Inside lysosomes, propeptide is cleaved and converts procathepsins to mature cathepsins, which are active. This activation process includes autoactivation or transactivation or both. In autoactivation, the pH of lysosome helps in propeptide cleavage by the same enzymes catalytic site, while in transactivation process, this needs the help of other proteases (Yadati et al., 2020). Fig. 35.1 represents the cathepsins synthesis in normal cell.

In malignancies, the proteolytic activity of cathepsins is abnormally active, which leads to the degradation of ECM components causing the invasion of tumor cells, which may leads to malignant tumor formation (Olson and Joyce, 2015). In many malignancies such as breast, colon, ovary, bladder, skin, head, and neck, the level of extracellular cathepsins is highly expressed (Mohamed and Sloane, 2006). Generally, in oncogenesis, cathepsins are released in to the extracellular space when lysosomes are transported from the perinuclear space to the periphery of the cell and the acidification of the extracellular space helps in enzyme activity. In the extracellular space, cathepsins also promote the interruption of insulin-like growth factor 2 receptor that helps in the transport of cathepsin protein to lysosomes. This results in the overexpression of cathepsins in tumor cells. Depending on the tumor type and location, the significance of a specific cathepsin can vary. Whereas, cathepsins in cancer cells and cancer-associated leukocytes, fibroblasts, osteoclasts, and myoepithelial cell have an

TABLE 35.1 Types of cathepsins with functions.

Cathepsin	Function
Aspartate proteases	
Cathepsin D	Lysosomal protein degradation
Cathepsin E	Presentation of antigen
Cysteine protease	
Cathepsin B	Antigen processing, catabolism of protein
Cathepsin C	Hydrolyze dipeptide esters, and amides
Cathepsin F	Presentation of antigen
Cathepsin H	Endopeptidase activity
Cathepsin K	Remodeling bone, lysis of collagen
Cathepsin L1	Presentation of antigen, keratinocyte differentiation
Cathepsin L2/V	Processing of the MHC class II molecules, release of antigenic peptides
Cathepsin O	Cellular protein degradation and turnover
Cathepsin S	Protein degradation, invariant chain II degradation
Cathepsin W	Unknown
Serine carboxypeptidase	
Cathepsin A	Autophagy, platelet activation
Cathepsin G	Degradation of extracellular matrix, inflammation

efficient power to treat cancer. In an acidic environment, cathepsins are active, and based on the type of cells, their expression level varies (Nicotra et al., 2010). Table 35.2 represents the types of cancer where cathepsins are overexpressing. These are very efficient enzyme category that has gained a lot of attention nowadays for nanoscale drug delivery in cancer.

Different cathepsins have different properties for hydrolyzing various bonds; in case of cysteine protease, cathepsin C can hydrolyze, amides, anilides, and beta-naphthylamides (Radzey et al., 2013; Minarowska et al., 2012). While cathepsin F can competently reduce the MHC class II-associated invariant (Ii) chain, cathepsin H is an aminopeptidase with limited endopeptidase activity. Likewise, cathepsin K is mainly involved in osteoclasts' degradation and has a vital role in osteoporosis (Shi et al., 2000; Vasiljeva et al., 2003; Dai et al., 2020). In B cells, cathepsin S has an important role for the active Ii chain degradation, which is very important for rendering the class II molecules to bind peptides. In immune cells, the expression of cathepsin X helps in the phagocytosis and immune response regulation (Riese et al., 1996; Obermajer et al., 2008). Cathepsin L is involved in processes such as

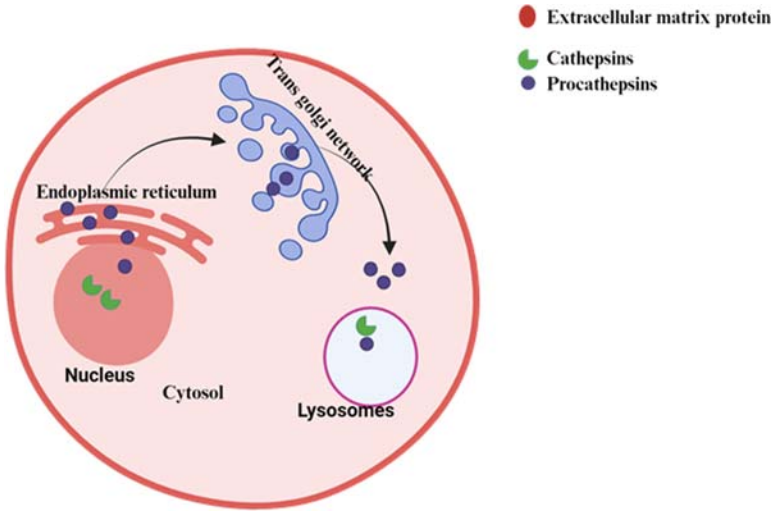

FIGURE 35.1 Synthesis of cathepsins in normal cells.

TABLE 35.2 Different cathepsins expression in various cancer types.

Cathepsin	Location	Site of tumor	References
Cathepsin K	Extracellular	Breast, bone	Littlewood-Evans et al. (1997)
Cathepsin B	Extracellular and pericellular under pathological conditions	Breast, cervix, colon, colorectal, gastric, head and neck, liver, lung, melanoma, ovarian, pancreatic, prostate, thyroid	Dheer et al. (2019)
Cathepsin L	Endopeptidase	Breast, colorectal	Sudhan et al. (2016), Poreba et al. (2018)
Cathepsin E	Endosomal structures, ER, Golgi bodies	Cervical, gastric, lung, pancreas adenocarcinomas	Konno-Shimizu et al. (2013), O'Donoghue et al. (2016), Zaidi and Kalbacher (2008)

cell cycle regulation and antigen presentation and also helps in the development of natural killer cells (Turk et al., 2012).

Cathepsin A, a serine carboxypeptidase, plays a crucial role in the formation of elastic fibers; it complexes with enzymes beta-galactosidase and neuraminidase and is involved in autophagy (Bonten et al., 1995). S1 class of serine protease cathepsin G plays a vital role in murine microglia, mDC1, mDC2, and B cells (Burster et al., 2010). At the same time, cathepsin D is essential in controlling the cell and thus maintains tissue homeostasis (Yadati et al., 2020). Similarly, cathepsin E is chiefly seen in the immune system, antigen-presenting cells, and human M cells (Yamamoto et al., 2012). In human tissues, cathepsin Z is expressed in intracellular proteolysis (Santamaría et al., 1998; Zaidi and Kalbacher, 2008).

4. Cathepsin-sensitive nanoparticles for malignant tumors

Most of the studies reported so far is about how nanoparticles are delivering the cytotoxic drugs to the tumor environment. For this purpose, the drug is encapsulated/incorporated to the nanoparticles of choice, which may be lipids, proteins, polymers, or viruses that are endocytosed by the cancer cells. The nanoparticles are retained in and around the tumor cells with cell-interactive ligands such as proteins, peptides antibodies, or other receptor ligands. Several groups have developed many cathepsin-sensitive nanomaterials against different tumors/cancer conditions. Many researchers have reported the development of therapeutic approaches that are specifically targeting cancer cells, by expressing cathepsin B with the help of nanoparticles. Cathepsin B functions as an endo- and exopeptidase, which are usually present in macrophages, pancreas, renal tubules, hepatocytes, salivary glands, gastrointestinal epithelium, stratified squamous epithelium, transitional epithelium, central and peripheral neuronal cell bodies, and all endocrine organs (Mort et al., 1997; Howie et al., 1985). Cathepsin B−targeted bioenzyme acts as a specific tumor biomarker, which can activate the prodrugs at the sites of tumor. For the study, Dox is used as a prodrug with small peptides conjugation, as conjugation can reduce the DNA intercalating action of uncleaved-Dox in targeted tumor cells. Shim et al. (2019) in their work reported the synthesis of cathepsin B−cleavable peptide (Phe-Arg-Arg-Gly; FRRG) −conjugated Dox prodrug (FRRG-Dox), which is a carrier-free nanoparticle with 213 nm size and has a stable structure, in aqueous conditions. In the targeted tumor tissues, these peptides can deliver through EPR effect. At the sites of tumor, the FRRG-Dox nanoparticles cause extreme toxicity by cathepsin B cleavage of prodrugs in tumor cells. But in normal cells the nanoparticles have no specific toxicity due to distinctively reduced cathepsin B expression. Here, the FRRG-Dox nanoparticles target tumor cells in mice bearing with human colon adenocarcinoma (HT-29). The results showed the therapeutic potential of FRRG-Dox nanoparticles was meticulously associated with cathepsin B expression in tissues with tumor tissues. In normal cells, compared with free Dox-treatment, the nonspecific toxicities, such as cell death and cardiotoxicity, were much less in FRRG-Dox nanoparticles treatment (Shim et al., 2019).

Another study by de la Torre et al. (2014) showed the controlled release of drug safranin O (S1−P) or Dox (S2−P) from the mesoporous silica nanoparticles capped with peptide in cathepsin B−expressing cancer cells. The cells with cathepsin B and cells lacking cathepsin B expression were studied. The cells with cathepsin B selectively hydrolyzed the capping peptide, and the study showed that the release of S1−P or S2−P only takes place when cathepsin B is active. Whereas the cells treated with S2−P showed reduce cell viability due to the hydrolysis of capping peptide by cathepsin B stated the use of peptide capped mesoporous silica nanoparticles as new therapeutic agent against cancer (de la Torre et al., 2014). The studies by Tarassoli et al. (2017) reported the synthesis and characterization of indocyanine green (ICG)−containing polyglutamate (PGA) nanoparticles. In this study, cathepsin B digestion of nanoparticles decreased particle size and increased ICG cellular uptake. The presence of cathepsin B ICG-incorporated PGA nanoparticles provides NIR-absorbing agents with time-dependent altered optical properties and cytotoxicity. The study results showed that this therapeutic strategy can help treat superficial malignancies,

such as head and neck tumors. Another study by Eldar-Boock et al. (2011) reported the synthesis of a polyglutamic acid (PGA)-PTX-E−[c(RGDfK)2] nanoscaled conjugate for tumor targeting. The PGA is biodegradable by cathepsin expressed in the tumor tissue. This helps in the release of drugs in a time-dependent manner in tumor tissues. In addition, the targeting ligand incorporation to the cells with integrin expression can lead to antiangiogenesis to reduce multidrug resistance.

Organic mesoporous silica nanoparticles immobilized with rotaxanes were developed for cathepsin B−induced targeted delivery to tumor, which is loaded with Dox (Cheng et al., 2015). This HeLa cells effectually internalized the novel nanocarrier by integrins-mediated targeting, and Dox was released through enzymatic digestion of GFLG peptide due to the overexpression of cathepsin B in late endosomes and lysosomes of tumor cells. In another study by Yang et al. (2011), by the method of click chemistry, Dox was conjugated to an enzyme-cleavable peptide precursor that was linked to silica-coated magnetic nanoparticles. These nanocarriers showed efficient drug release into the site bearing tumor with prominent expression of cathepsin, thus helping the imaging of the cancer cells, whereas the synthesized material showed lesser cytotoxicity to other cells without enzyme expression. In drug delivery studies, the application of dendrimers had paid great attention as these are monodispersed and significantly branched 3D macromolecules. The study by Li et al. (2014) showed the synthesis of peptide dendritic Dox drug conjugate-based nanoparticles, which are recently developed as a potential candidate for drug delivery vehicle in 4T1 murine breast cancer model. In this study, cathepsin B was used for the selective degradation of the dendritic shell to generate sustained Dox release near the tumor cells. This nanocarrier cleavage by the enzymatic process may be seen as a new method for controlled DDS. This approach will lead to enhanced antitumor potency.

In lipid-based DDS, most of the recent researches showed that the lipid-based DDS can be used as lipidic nanocarriers with loaded drugs or lipidic prodrug nanocarriers (Mura et al., 2015; Kalepu et al., 2013). Studies on cathepsin-sensitive lipidic DDS are relatively rare. One of the studies reported that when a lipidated cathepsin B inhibitor (NS-629) was incorporated into a liposome bilayer, this helps in the targeting and internalization into the tumor cells and the stromal cells in ex vivo and in vivo models (Mikhaylov et al., 2014). In contrast, DDSs based on proteins or peptides are widely used because of their compatibility. Studies on small interfering RNA (siRNA) delivery with cathepsin sensitivity have been reported to have anticancer activity. Gibori et al. (2018) showed the synthesis of polyglutamate amine (APA) nanocarriers with miRNA and siRNA polyplexes, which showed significant aggregation in tumor cells in pancreas. Their study reported that in the presence of cathepsin B, APA-containing polyplex releases the miRNA. The study by Kulsharova et al. (2013) reported the synthesis of gelatin nanoparticles−coated cathepsin D-specific peptides, and this acts as a vehicle for the targeted drug delivery of Dox in breast malignancy. In this work, cathepsin D caused the release of Dox by degrading the peptide layer of the nanoparticles. In vitro and in vivo conditions, the fabricated nanoparticles were localized in the cancer models and showed the distribution of nanoparticles in the mice models. The finding supports that cathepsin D-coated nanoparticles were highly encouraged in targeted therapy in breast cancer.

5. Cathepsin-sensitive nanosystems for imaging

Currently, a wide range of nanomaterials are used in medical research as probes for imaging that can be activated by cathepsins; by enzymatic cleavage, the detectable signals are produced from the probes. These probes are used for the detection of disease by targeted study through signals, which are easily detectable with nonspecific degradation and easy clearance from the body, which are done mainly through nuclear imaging (PET, SPECT, and CT), optical/fluorescence imaging, magnetic resonance imaging (MRI), and more with accurate signaling (Weissleder and Pittet, 2008; Willmann et al., 2008; Zhang et al., 2015). In cancer research, these are used for improving patient care like diagnosis and earlier detection of the disease and response monitoring of patient. A few researches have been reported over the recent decades, discovering the synthesis of cathepsin-sensitive probes with potential clinical applications. Whitley et al. (2016) reported that a near-infrared FRET-based probe with cathepsin S sensitivity, which is in clinical trials (phase 1), has medical applications in soft tissue sarcoma and breast cancer. Lock et al. (2013) reported the synthesis and design of nanobeacons, which comprise hydrophobic and hydrophilic domains. The hydrophobic domains is with fluorescent dye 5-carboxyfluorescein (5-FAM) and a black hole quencher (BHQ-1), whereas hydrophilic domain is with an HIV-1 derived cell−penetrating peptide sequence (Tat48−60). In this, the charged residues permit the nanobeacons to respond based on the pH change of the environment. A cathepsin B−cleavable linker was attached to the fluorophore and quencher in this study. This study was effective in MCF-7 human breast cancer cells in vitro and showed the lysosomal localization of the fluorescent signals. Another study by Sun et al. (2019) reported the synthesis of pH-dependent and cathepsin B−activable $CaCO_3$ nanoprobe for targeted in vivo tumor imaging. The $CaCO_3$ nanoprobe showed good stability and imaging ability in tumor cells in low pH. This helps in inhibition of tumor growth in in vivo studies. Another group developed a cathepsin B−specific metabolic precursor, which can yield unnatural glycans containing azide groups on the cell surface of the tumor. Cathepsin B−specific cleavable substrate (KGRR) conjugated with triacetylated N-azidoacetyl-D-mannosamine (RR-SAc3ManNAz) was synthesized in their study. Furthermore, on the surface of the tumor cells, unnatural glycans are present and were conjugated with near-infrared fluorescence (NIRF) dye-labeled molecules in vitro and in vivo models. The study results showed that the RR-S-Ac3ManNAz is promising for tumor-specific imaging or drug delivery studies.

6. Conclusions and future perspectives

Cathepsins are an important target for designing advanced nanoparticle-based DDS in cancer therapy. Most of the new findings on cathepsin-responsive nanodelivery systems will help us to comprehend its role, and also, it's targeting efficiency on tumor bearing cells, albeit most of mechanistic role of this cathepsin-responsive nanodelivery is yet to be explored. Currently, cathepsin-based drug delivery agents have acquired great attention in biomedical field as they lead to improve the therapeutic and imaging properties.

Effective drug delivery to the site of cancer is still a question to be explored more and an important pharmaceutical issue in the field of cancer theranostics. There are several limitations to enzyme-responsive nano-DDS that affects its robustness such as the complexity and variability of tumor-associated proteases, limited enzyme-substrate incompatibility, expected toxicity, biocompatibility of nanoformulations, etc. This demands thorough and comprehensive research to enlighten further its usefulness. The future of cathepsin-sensitive nanoparticle-based DDS reported to date looks promising than ever and needs further clinical trials to explore its potential use in cancer research.

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Pathophysiological Aspects of Proteases in Cancer

Edited by

Sajal Chakraborti, Srijit Das, Cheorl-Ho Kim

Pathophysiological Aspects of Proteases in Cancer provides a comprehensive overview of the multifaceted field of proteases in the cellular environment and focuses on the recently elucidated functions of complex proteolytic systems in physiology and pathophysiology. In 35 chapters, this book gives insight into the general aspects of the role of proteases in cancer, their role as diagnostics and prognostics biomarkers, their impact on chemoresistance and the future cutting edge technologies like cocktail therapies and CyberKnife technology. Given the breadth and depth of information covered in the respective contributions, this book will be immensely useful for researchers in oncology and working to identify targets for drug development. Multidisciplinary in scope, this book bridges the gap between fundamental and translational research, with applications in the biomedical and pharmaceutical industry, making it a thought-provoking read for basic and applied scientists engaged in biomedical research.

Key Features

- Offers insight into the spectrum of proteases, protease inhibitors, and protease interactors involved at the cellular and molecular level in cancer development
- Gives a comprehensive overview of the role of proteases in modulating cellular metabolism and chemoresistance from the early stages of cancer development and metastasis to its end point
- Provides a multidisciplinary approach demonstrating the biochemical and signal transduction mechanisms associated with dysregulation of proteases, leading to the manifestation of various diseases
- Highlights the roles and regulation of different types of proteases, as well as their synthetic and endogenous inhibitors
- Bridges the gap between fundamental and translational research, with applications in the biomedical and pharmaceutical industry

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He has participated in various National & International seminars, presented more than 10 papers & published articles in various Journals. He has good clinical, teaching experience along with Yoga expertise.

He has put an sincere effort to make the subject an interesting study for the Ayurveda fraternity.

About the book

The text containing 5 topics/Chapters. First chapter deals in detail description about Shukra as per Ayurveda classics. 2nd chapter deals in detail description about Semen as per contemporary science. 3rd chapter deals in detail description about Shukrotpatti as per Ayurveda classics. 4th chapter deals in detail description about Spermatogenesis as per contemporary science. 5th chapter deals in detail description about correlation between Spermatogenesis & Shukrotpatti.

This is the humble effort of the writer which is contribution to the field of Aurveda. Hope the text will be accepted to Ayurveda fraternity.

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Spermatogenesis correlation in Ayurveda • Dr. Praveen N. Bhirdi

Spermatogenesis correlation in Ayurveda

Dr. Praveen N. Bhirdi

Futuristic Trends in

MEDICAL SCIENCES

Volume 3, Book 10, 2024, IIP Series

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PREFACE

Book series on Medical Science gives the opportunity to students and doctors from all over the world to publish their research work in a set of Preclinical sciences, Internal medicine, Surgery and Public Health. This book series aim to inspire innovation and promote academic quality through outstanding publications of scientists and doctors. It also provides a premier interdisciplinary platform for researchers, practitioners, and educators to publish the most recent innovations, trends, and concerns as well as practical challenges encountered and solutions adopted in the fields of Medical Science. It also provides a remarkable opportunity for the academic, research and doctors communities to address new challenges and share solutions and discuss future research directions in the below field but not limited to

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APPLICATION OF AGADA PRINCIPLES IN THE MANAGEMENT OF SKIN DISORDERS

Abstract

The Ayurvedic concept of Dooshivisha holds good for the present-day food and lifestyle where exposure to chemicals and toxins is unavoidable and even the food chain is entangled with pesticides, fertilizers and various processing agents. These low potent poisons produce systemic and metabolic diseases when they get precipitating factors, among which skin diseases are most common.

The beauty of Agadatantra lies in its unique way of approaching the diseases, focusing mainly on the Nidaanaas while treating. These may be any of the factors that the person is getting exposed since the Garbhavastha. The etiological diagnosis also rules out the skin manifestations due to Aabharana visha, Jangama visha and Sthaavara visha which helps in treating the disease effectively saving time and unnecessary intake of excess medicines. So the rationality lies in the selection of agadaas and shamanoushadhis in the management of these diseases. Few of such cases along with their causes,
diagnosis and management are discussed here to understand the concepts of
Agada and their application in managing skin disorders.

Keywords: Dooshivisha, Garavisha, Agada, Skin diseases

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Chemicals and toxins in present day food and lifestyle including environment is undeniable fact which causes assault to body. Food chain is entangled with these residual or altered pesticides and fertilizers may it be vegetables, grains, fruits to meat (broilers) which is dosed with several drugs and cattle with hormones for high yield of milk. Food is made attractive with additives, artificial colors and preservatives in the name of permissible limit but goes as unidentifiable etiology for skin, immune and many other disorders. To add on, anirrational use of medicaments and environment painted with different toxins has undesirable effect on health. This scenario has been mentioned in Ayurveda, where in low potent poisons of artificial origin called as Garavisha gain entry to body and act as Dushivisha.

Dushivisha is state where any low potent poison (vegetable, mineral and animal origin)can stay in the body for several years and cause diseases on getting precipitating factors.Dushivishaaccumulated in Amashaya causes kapha&vaata disorders and in pakwaashaya causesvaata& pitta disorders which may then lead toorgan specificity, for example by affecting shukracausesbeeja dosha, by affecting Rakta causes Kushta and so on. It can be inferred that Dushivisha patients may suffer from liver disorders, GI disorders, urinary disorders or reproductive disorders etc depending on the nature and type of poison. It may also manifest as an allergic condition mostly of skin or respiratory system. Most of the occupational disorders seen in persons working in places where chances of exposure to chemicals, heavy metals etc. suffer from several disorders which can be considered and treated effectively as dushivishajavikaaras. Prolonged and injudicious medication may also develop as dushivisha.

I. ASSESSMENT OF EXPOSURE TO ETIOLOGY:

In the diagnosis of any disease usually three kinds of diagnosis are made.

1. Clinical diagnosis
2. Morphological diagnosis
3. Etiological diagnosis

Clinical diagnosis refers to the assessment of clinical presentation and analysis of dosha involvement and dominance. Morphological diagnosis refers to understanding the extent of the disease, where one can assess the part affected and the duration etc. Etiological diagnosis is the search for causative factors of the disease and it should be done very carefully, as the selection of treatment largely depends on this and there by the success of the treatment.

In any skin condition we can make etiological diagnosis in two headings.

- Exposure before birth
- Exposure after birth
- Local exposure
- Systemic exposure

1. **Exposure before birth:** These factors can be treated as Garbhopaghatakara bhavas, where it is said that if mother gets exposed to disease specific etiologies before

conception or during fertilization or in early post fertilization period, the offspring will suffer from those specific diseases or will get Kha vaigunya(susceptibility) suitable for specific diseases. Here not only mother, but father is also responsible by contributing (like) dushta shukra. Both parents play important role in the causation of diseases to their children. To establish this we will discuss two cases.

2. **Case 01:** A 12 years male child approached with the complaints of multiple skin lesions like bullae, vesicles and erythema all over the body gradually in episodic fashion since one and half month after birth. History dissection said child's elder siblings are apparently healthy, no specific Antenatal history by mother that was specific to contribute for the etiology. But a formal history from paternal side gave an edge to existing condition of child. Father was diagnosed as epileptic 6-8 months prior to conception of this child and underwent anti-epileptic and anti-anxiolytic therapy. So it may be derived that patient's condition can be caused by the shukradusti due to drug.

Figure: 01

3. **Case 02:** A 3 years female child was brought with complaints of vesicles and pustules associated with oozing, itching and general weakness. It was told that the baby started developing pustular lesions over abdomen and back between 15th to 20thdays of birth. Gradually severity increased and availed contemporary treatment regularly but the complaints were recurring veryoften. History reveled that there was no specific family element of skin diseases in both mother and father, even the elder child of couple was

healthy. Antenatal history of mother lauded that during her 3rd month;shemet with an accident and had fracture of right tibia. She underwent surgical repair and treatment with analgesics and antibiotics for more than two and half months. So it may be considered that the exposure of garbha to drugs led to the present pathology.

Figure: 02

4. **Exposure after birth:** When a patient comes with particular skin manifestations, the dermatologist should always search for the type of exposure so that one can plan the treatment as well as do's and don'ts.

5. **Local exposure:** Very often it is believed in the practice that some chemicals or substances produce skin diseases as a result of allergy and the patient is treated symptomatically. Hence asked to avoid the substances/chemicals to which he/she is allergic. Scientifically this should not happen as we understand the allergy caused by chemicals/substances is not exactly by itself, rather it is because of the environment in one's own body, which is brought about by various etiological factors. When we see the indications of Bhallataka, it is suitable for Vaata-Kapha dominant diseases and people.

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While same is contraindicated in Pitta dominant disease and Pitta prakruti people, because it acts adversely or like visha to them. This is not because for Bhallataka, but it is the body's composition or environment that is not suitable for Bhallataka. Hence people develop complications, but at the end Bhallataka is regarded as drug causing adverse effects.

6. **Systemic Exposure:** As discussed earlier the present day etiologies namely Dushivisha and garavisha that are gaining entry into body through toxicated food, water and air etc affect the body's ability to prevent disease manifestation, which is brought about by Oja, which is the prime target of any type of Visha. Individuals ability to adapt for changes in food, climate etc is altered/affected and lands into conditionof Vikaravighataabhava, this makes even the minimal etiologies to cause disorders and in this context skin diseases. This systemic exposure itself determines the effect of local exposure and hence the severity of the skin manifestations.

7. **Etiological diagnosis** can also be made by understanding morphological findings of Skin manifestations as mentioned in the context of Paduka visha, Abharavishaetc. Even accessories like footwear's or jewelry if contaminated with chemical or poison can develop skin manifestation over the contact area like dorsum of feet or wrist etc.This goes on to say that wherever the offending agent comes in contact with skin there it will cause skin manifestations.

If low potent toxins either natural or artificial origin vitiates blood then these will cause skin manifestations presenting as Kitibha, Khota etc.

These poisons have specificity in causation of various diseases like some cause Psychological/Psychiatric disorders, few cause speech disorders, others cause GI disorders, Reproductive disorders etc. This gives clear picture about their organ specificity. It is said if they affectshukra, then will cause diseases in offspring, if affect Rakta then will cause Kusta(skin manifestations).

These clinical manifestations explained in the context of Dushivisha or Garavisha cannot be seen in all types of toxin/poison exposures but depends on specific toxin and its affinity to tissue. Many scientific studies have shown that exposure to pesticides in low doses is common and this can cause neurological and reproductive disorders.

Similarly another study says that Daughters whose mothers took DES (Diethylstilbestrol is a synthetic estrogen) (about 1 million or more between 1960 and 1970) suffer reproductive organ dysfunction, abnormal pregnancies, a reduction in fertility, immune system disorders, and periods of depression.

As it is very clear here that some low potent poisons affect shukra thengarbhadeveloped by that shukra will develop diseases either in the intrauterine life or after birth. Similarly skin manifestations can occur by affecting rakta dhatu. Another study of environmental factors like pollution exposure in preconceptional, prenatal and postnatal period showed that chemicals can cause allergic disorders like eczema in offsprings.

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Skin manifestations not only caused by Sthavara visha (poisons of vegetable and mineral origin) but even several venoms (poisons of animal origin) also cause skin manifestations, as most of insect bites and stings mainly present with skin manifestations. To highlight this one can consider Loothavisha clinical presentations, as it is explained that Loothavisha presents with wheels, rashes, pustules, vesciles, discolorations, oozing, itching, fever, etc. It is also explained that the discharge from these skin manifestations produces new lesions on the healthy parts of the body when it comes in contact.

II. TREATMENT

Etiological diagnosis is important in successful management of any skin manifestation caused by poison because selection of etiologic specific Agadas or medicaments depends on it. Once the etiological diagnosis is established, clinical diagnosis will help to assess the dominance of dosha and treatment protocol.

Protocol framed then will be falling like Agada or Vishaharaoushadhi for etiology and Shamana for dosha modulation which includes kashaya, churna and external modalities like pariseka and tail application.

But the principle of protocol should be and carefully framed with medicines having vishara property or indicated in visha conditions (ex: patolokaturohinyadikashaya) or least they should not be contraindicated in visha conditions.

In general the following Doshivishachikitsa is adapted when skin manifestations are caused by the exposure to Low potent poisons or toxins. Dushivisha patients should be administeredSwedana followed by Vamana or virechana or both[10]. As such there is no mention of Snehapaana for Dushivisha, but depending on the clinical presentations one has to decide whether snehapana is to be done or not.

If the patient presents with Vaata dominant lakshanas then snehapaana is to be done,if the patient presents with increased Kleda (kaphautklesha) and haveuttamabala (strength), then one can do abhyanga and swedana followed by suitable shodhana i.e. vamana. Same is followed for Pitta utklesha conditions and virechana is done. Also in this context only Vamana and Virechana are mentioned, but by considering the general treatment principles of Kushta one can opt for Raktamokshana.

If the patient presents with Vaata dominant chronic skin manifestation with reduced bodily strength then one should choose to do Snehapaana by selecting appropriate ghrita (internally taila is contraindicated in visha condition as it potentiates visha), then abyanaga and swedana followed by appropriate shodhana. Vamana is adopted inKapha dominant and Virechanain Pitta or Vaata dominance. Commonest used ghritas are Kalyanakaghrita, Tiktakaghritaand Mahatiktakaghrita, but guggulu containing ghritas are usually avoided as the teekshna properties of guggulu may aggravate the condition by potentiating low potent visha.

Acute skin manifestations as a result of exposure to Visha are very often cured or managed by shaman therapy alone and/or along with Sadhyahshodhana like Vamana, Virechan, Raktamokshana whichever is suitable for the presenting condition. In acute

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manifestations Snehapana is not done, in such condition sadhyahshodanais done as the doshas will be in Utklishtaavastha and they can be easily eliminated by approptiateshodhana. In such conditions if one plans for Snehapana which takes 3 to 7 days, during that period itself the disease may get aggravated and management becomes difficult.

III. LET US DISCUSS FEW CASES

1. **Case number 01:** 18 years male approached with complaints of pustules, discharge, local odema, discoloration and severe itching over dorsum of both foot over a month. History revealed that it started after having new footwear and diagnosed as paduka visha (footwear dermatitis). This was considered as vishaja and complaints showedsannipatajalakshana with pitta-kaphja dominance (pustules, discharge, local odema and itching). Internally Bilwadi agada and Patolakaturohinyadikashaya was used while externally pariseka with Lodrasevyadikashaya followed by Nalpamaraditail application was done for 15 days. Later to this treatment nearly all complaints subsided except vaatalakshana (dryness & hyperpigmentation), hence for this Kalyanakaghruta was advised at shaman dose of 10 ml on empty stomach, Dushivishari agada twice daily after food with honey and Eladi tail for external application for 15 days. One month of vishaharachikitsa relieved all complaints.

Before treatment **After treatment**

Figure: 03

2. **Case Number 02:** A female aged 21 yrs approached with the complaints of recurrent skin peeling as very thin layers over face, trunk and extremities, associated with itching and discolouration from the age of nine. She experienced such peels not less than 10 episodes per year. Detailed history gave an incident of infection at age of eight and treated with injection penicillin for which she developed instant allergic urticarial rashes all over the body and was managed immediately by conventional medication, but she started to develop peeling of skin on most parts of the body which gradually increased. For this sheunderwent conventional treatments but was futile. She also revealed that it gets aggravated in rainy season, exposure to cold climate, eating cold things, food having additives (artificial food colours, taste enhancers, flavours etc.) and by taking antipyretics and analgesics. Based on the history, etiologically it was diagnosed as vaata pitta dominant (skin peeling and discoloration) skin manifestation caused by Dooshivisha (aggravates on exposure to vishavardhaka bhava as explained above). In contemporary medicine the condition was entitled as Erythema Annulare Centrifugum. This patient was

treated successfully on the basis of Dushivisha and for last 07 yrs patient is completely free from the said complaints even after exposing to above detailed aggravating factors.The patient was undertaken for virechan with Tiktakaghritasnehapana, Eladi tailaabhyanaga followed by swedana. Later she was kept on Dushivishari agada with honey and Haridrakhanda thrice a day for a period of six months with 20 days medication and 10 days gap during entire 6months.

Before Treatment **After Treatment**

Figure: 04

3. **Case Number 03:** A male patient aged 42yrs farmer by occupation came with the complaints of vesicles over dorsum of left foot around the ankle extending upto midleg with serous discharge, pain, itching and swelling over left lower limb. This was associated with loss of sensation over left foot and first three toes since 8 months. Similar lesions over medial aspect of right leg with mild itching and oozing since own week. Notable incidence was expressed by the patient saying that within a day new lesions would appear wherever the discharge of vesicles came in contact with fresh skin and hence always tried to keep the lesions dry. Etiology of unknown bite and morphological presentation diagnosis was made as Loota visha (wherever the discharge from these skin manifestations comes in contact produces new lesions-A.Hru.U.37/58) while conventional diagnosis was Erythema Nodosum leprosum and treated priorly with Dapsone, Rifampicin and clofazimine for more than 3months and there was no relief from the complaints, instead they increased.

Dashanga agada indicated in keetavisha and Patolakaturohinyadi Kashaya (15 ml) was given 4 times a day along with Triphalakashayapariseka followed with Parantyaditaila application externally twice daily for 08 days. Later to this when Pitta-kaphajalakshan came down (reduced discharge, itching and swelling) Dashanga agada was replaced by Bilwadi agada keeping other medications same for next week. Patient started to experience pain over the region of foot where loss of sensation was complained in first visit. After 2 visits Pitta-kaphjalakshana came down completely and treatment was continued with KalyanakaGhrita 10 ml on empty stomach, Bilwadi agada thrice daily

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after food internally and Eladi tail for (indicated for twakprasadana and kanduhara) external application for next 15 days. Post to this Loota vishachikithsa normalcy of sensation over foot was restored and relief of other complaints.

Figure: 05

After Treatment

4. **Case Number 04:** A male patient aged 39 working as tourist driver approached with sudden onset of redness, small reddish papules over both upper and lower extremities spreading up to lower abdomen, severe burning sensation, mild persistent fever and odema over both lower extremities since 4-5 days. The condition was associated with headache, thirst and generalized weakness. On local examination there were few necrotic circular lesions of size less than 0.5 cm suggesting of some insect bites. History directed towards an insect bite while touring the clients in Dandeli forest safari and by the same evening he developed above complaints which flared in 2-3 days. Consulted conventional physician but no relief was found and went on to be diagnosed as Leukocytoclastic vasculitis. As per Agadatantra the morphological and etiological diagnosis was Keeta visha and treated successively and successfully. As the lesions were Sannipatajawith Pittakaphapradhanata (burning sensation, skin peeling and odema) hence Patolakaturohinydaikashaya 10ml five times a day along with Dashanga agada four times a day internally and Erythaila (indicated in sarpavishachikitsa in kerala tradition) was given as external application for first two days. By day 3 of admission the complaints subsided (redness, odema and fever came down) and patient was taken for sadyovirechana with Avipattichurna, where patient had 7 vegas and once blood stained stool. Next morning complaints of redness, burning sensation, swelling and fever were disappeared except the vata dosha lakshana like multiple joints pain and kostarukshata (indicted by blood stained stool). For the vatalakshanwe adopted Tiktakaghruta 10ml once in the morning on empty stomach with hot water and Bilwadi agada thrice a day after food for next 03days along with Nalpamaraditaila for external application. After this course of medication patient become completely free form the complaints.

Before Treatment **Before Treatment** **After Treatment**

Figure: 06

5. **Case number 05:** A farmer by occupation aged 50 years complained of raised erythematic circular lesions all over body including oral and anal mucosa, itching, burning sensation, scaling with mixed presentation of dry and pus formation, multiple joint pain and generalized weakness since 3 years. Associated with constipation and loss of appetite. Patient's previous medical history was strong indication for development of existing condition. He had taken over the counter medication (self mediation) for his previous complaints of pustular acne lesions over face and back which actually again

aggravated the condition. For this he consulted local physician and received few oral and intravenous medications which after 4 days developed severe skin reactions/allergy as mentioned above. He neglected these for month and then experienced skin lesions all over the body. Consultation at peripheral center diagnosed condition as Erythema Multiformae a condition caused due to hyper sensitivity reaction. According to Ayurveda the condition was diagnosed as Dooshivishajanya Pitta-Kaphaja Kusta (itching, burning sensation and erythematic circular lesions) and Dooshivishachikitsa was adopted. The patient was first treated with Patolakaturohinyadikashaya 15ml four times along Dushivishari agada 1gm four times a day internally and Triphalakashayaseka and Nalpamaraditaila as external modalities for 5 days. After developing good appetite patient was taken for Virechanawith Tiktakaghritasenhapana for 5days followed by abhyanga with Nalpamaraditaila and Triphalakashayaseka (as Sweda) for three days and virechana was done by giving Trivrutleha. After shodana the patient was put Nimbadikashaya and Bilwadi agada internally (for pitta-kapha shaman and raktaprasadana) and Nalpamaraditaila for external application for 15 days. By the end of follow up medication more than 60% relief was seen. Once again virechana was done with same protocol and same medicines were continued with addition of Tiktakaghritha (For vata shaman and ojavardhan) 10ml on empty stomach with hot water for next 20days. Since then patient is completely free from all the complaints and has not developed any further allergy to conventional medicines that he took for common illnesses thereafter till date.

Before Treatment **Before Treatment** **Before Treatment**

Figure: 07

Before Treatment

After Treatment

After Treatment

After Treatment

IV. RATIONALITY OF SELECTION OF AGADAS & SHAMANA FORMULATIONS

Management of any disease having poisons or toxins as the causative factors irrespective of high potency or low potency one should give importance to Oja or Bala , as poisons are told to act adversely over it. Hence treatment not only includes correcting disease but to nurture oja. As a reason one has to include all dosage forms and drugs having vishahara property which can protect oja and increase bala.

In skin diseases commonly prescribed Agadas are Dooshivishari agada, Bilwadi Agada and Dashanga Agada. Considering the ingredients of these mentioned agadas it can be said that Dooshivishari agada is Soumya yoga, Bilwadi agada is moderately teekshna yoga and Dashanga agada is most Teekshna among the three agadas mentioned here.

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Generaly it is thought that when skin conditions are severe in nature then soumyayogas are prescribed or chosen, but on contrary in skin manifestations caused due to poison or toxin exposure, which are acute and severe in nature one should give teekshnayogas. For example, Loothavisha which is Pitta dominant tridoshaja condition presenting with pitta pradhanalakshanas like srava and dahaetc, agada used is Dashanga agada. This is teekshna among mentioned three agadas, though independently the ingredients of this formulation are said to be Pitta vardhaka, here by virtue of its Prabhava it reduces the clinical manifestations of visha. Whereas for modulating the dosha, kashayas are given along with any agada.

Kashayas can be chosen as explained here, when there is increased Kleda and Srava we can chosePatolakaturohinyadikashaya along with the agada, if burning sensation is more then Chandana Parpatakadikashaya is opted, if jwara is associated then Amritottarakashaya is to be given, if Kleda and Kandu are dominating then Aragwadadi Kashaya is to be given, in the same way if Shotha is dominating then one can opt for Punarnavadikashaya. In this way along with agada, suitable shaman yogas can be selected, even more than one kashaya or shamana yoga can also be given.

Clinically Dooshivishari agada has been found effective in chronic skin manifestations with less pitta and kapha dominance, like in Urticaria, Chronic eczema, dermatitis, and other allergic skin manifestations.

Bilwadi agada is found to be effective in pitta dominant conditions even with discharge, itching, fever and swelling. It can be effectively used in vesiculo bullous presentations like Herpes, Acute dermatitis, Eczema, bites and stings induced skin manifestations etc. Bilwadi agada and Dashanga agada have also been found effective in preventing secondary infections in skin diseases like cellulites etc.

Among externally used yogas, Lodrasevyadi yoga, explained in the context of Loothavisha is also used for external application as Lepa or kashaya Seka other than internal administration. It is seen that it prevents further spread of the lesions, reduces burning sensation, oozing, itching and helps for faster healing of the skin lesions. Panchavalkadi Kashaya which is also considered to have vishahara property has been used as seka in conditions having more Pitta dominance along with Kapha, this helps in reducing excessive srava, Daha, kandu and enhances the faster healing of skin manifestations. Triphalakashaya is the next most commonly used formulation. It is used as Seka in skin conditions presenting with mild kleda and srava.

After seka,tailas are used for external application and commonest is Nalpamaraditaila, because of its kashayapradhanatait is used inconditions associated with itching and mild oozing. Panchavalakaladitaila is used when there is more discharge and burning sensation. When severe Pitta dominance presentations are seen Paranthyaditaila, which is explained in the context of Mandalisarpadamshachikitsa of Prayogasamucchaya, is found to be every effective. In the same way Erythaila, an oil preparation used by Traditional Visha vaidyas of kerala, is used in skin manifestations which are severe in nature also caused by potent bites and stings. For Vaata or Vaatakaphaskin manifestations likedryness, discolouration and itching Eladi taila is to be used. Pitta-Kapha dominant skin manifestations after treatment

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come down as Vaata-kaphja or vaatjaand hence there also at end Eladi taila can be used for external application.

Usually at the end of the treatment when one feels thatall the skin manifestations have completely reduced, the patient should beadministered with ghritayogas like Kalyanakaghrita, Tikataghrita andMahatiktakaghrita. This ghrita administration can be done for a period of 15 days or 30 days or as per the yukti of Vaidya, duration can be decided.The selection of the ghrita is to made byconsidering the dosha dominance and the nature of the disease and also therasayana properties of the ghrita. Kalyanakaghrita is best used when there isvaata and raktadushti is seen, it helps to restore the bodily strength, curesVisha of all origins and also nurishes Oja. It helps for correction ofdiscolouration and also helps to overcome the mental stress that patient willbe having. Tiktakaghrita and Mahatiktakaghritaare best utilized in the Pitta dominantconditions, both help to subside the Pitta which might not have been eliminatedcompletely during shodhana. Even if the condition is managed by only agada&shamana therapy, there also these ghritas are used effectively to counterPitta&Vaata vitiation. These ghritas also prevent further vitiation ofPitta and Vaata, improve pigmentation, and prevents further infections andsimultaneously help to improve Bala and Oja.

One more important issue is the time of administration of medicines, classically repeated administration of medicines is advised for visha conditions and the same is followed in all skin manifestations caused due to exposure to Visha. While such administrations patient should be carefully observed periodically as clinical manifestations change faster and hence one should note those changes and shamanayogas can be revised or entire treatment protocol is to be revised. For example when Aragwadadikashaya is given with some agada in kleda and kandupradhana condition, after 10 to 15 doses of medication the presentation usually changes to Vaata-Pitta dominance and hence Guducchyadikashaya or any other Vaata-Pitta hara shaman yoga is to be administered. In the same way if Teekshna agada is given in the beginning after 10 to 15 doses that can also be changed to milder ones as explained above.

V. CONCLUSION

The etiological diagnosis plays important role in the management of any disease. Present day life style, food style and environment clearly defines that each and every disease has one or other toxic (Visha) etiology. Based on the type of visha and its nature one has to assess clinical presentation, nature of disease (acute, chronic etc.) and dosha presentation one has to select agadas along with suitable shaman yogas.

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Practical Handbook on BHAISHAJYA KALPANA

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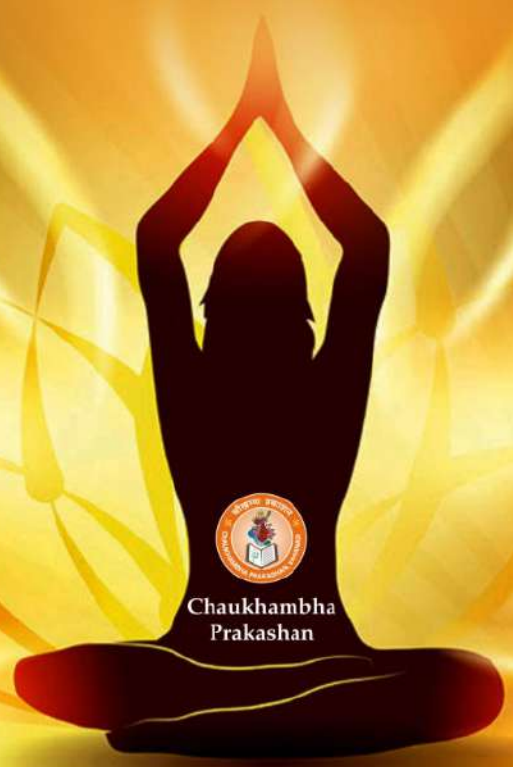

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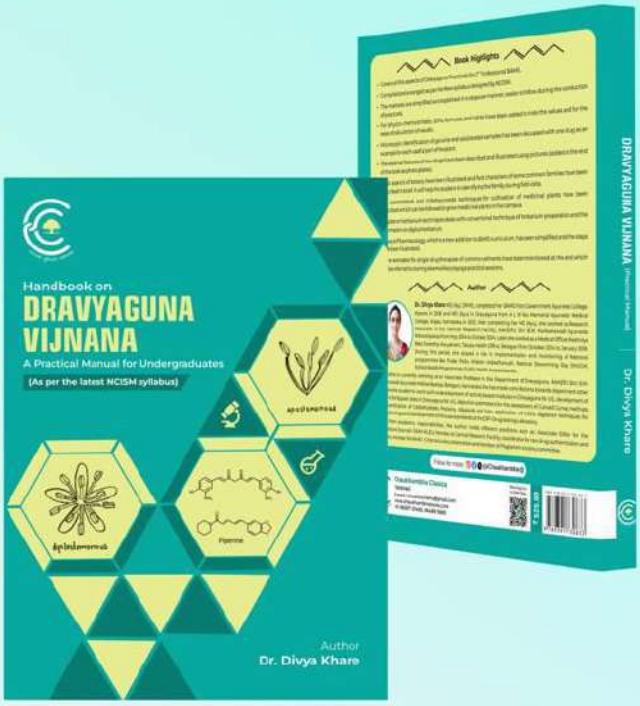

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- Network Pharmacology, which is a new addition to BAMS curriculum, has been simplified and the steps have been illustrated.
- Some examples for single drug therapies of common ailments have been mentioned at the end which can be referred to during ekamoolika prayoga practical sessions.

DATA INTEGRITY ISSUES, STATISTICAL TRENDS AND UTILIZATION OF ALCOA+ IN PHARMACEUTICAL INDUSTRY

Abstract

Background: Data integrity is critical to regulatory compliance, and the fundamental reason for 21 CFR Part 11 published by the U.S. Food and Drug Administration (FDA). Good documentation frames are an essential aspect of good manufacturing practises (GMP) in pharmaceutical enterprises because they play a vital part in the sanity of data preservation and its development, certifications, registrations, commodification, and life-cycle governance for pharmaceutical goods. Data integrity is a key concern in the regulated pharmaceutical sector due to observations and results due to inappropriate record keeping practices or data manipulation.

Main Body of the Abstract: In the present review article, data is collected from various online sources which includes articles, CFR and WHO guidelines and statistical data from Blogs. The articles have been thoroughly gathered and reviewed from a variety of databases and indexed journals.

Short Conclusion: An upsurge in data integrity breaches in 2019 led regulatory organisations such as the WHO and FDA to issue guidance on effective documentation practices. The present review article will help researchers to understand what is data integrity? Its importance in pharmaceutical industry, ways to reduce the data integrity issues by utilizing concept if ALCOA+ and recent WHO guidelines for data integrity issues.

Keywords: Data Integrity, 21 CFR Good Documentation Practices, Regulatory Bodies, ALCOA +, WHO

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I. INTRODUCTION

Data has always been an important part of in pharmaceutical manufacturing and research activities. The importance of data is growing tremendously in range of different factors in pharmaceutical manufacturing.During recent cGMP inspections FDA has observed numerous data integrity associated violations. Ensuring data integrity is vital component in pharma sector as it directly impacts the efficacy, safety and quality of drugs. Increasing data integrity issues, questions FDA's ability to protect public health. These data integrity issues have led regulatory bosies to carry out stringent actions which include warning letters, import alerts, form 483's and mutual agreement on termination[1]

Data integrity is a serious concern in the regulated pharmaceutical sector as a result of observations and outcomes of inadequate record keeping practises or data manipulation. This has led in multiple FDA warning letters along with data integrity guidelines provided by regulatory authorities like as the MHRA, WHO, FDA, and PIC/S, as well as industry associations.[2]

Every healthcare industry is concerned with Good Documentation Practises (GDP) within the organisation in order to lead the global marketplace of pharmaceuticals or medical devices. The procedures also involve extensive documentation to ensure that they are audit-ready and have traceable records. [5,6]

In recent years, healthcare sectors, particularly in India, have repeatedly received warning letters for data integrity flaws that have resulted in massive losses, for example [7]:

1. Regulations imposed by regulatory authorities such as the WHO, FDA, and others.
2. Due to lack of traceability, organisations are unable to respond to agencies
3. Constrained reputation in the market
4. Loss of client trust results in loss of business.
5. Questions on the marketed product regarding authenticity
6. Major deprivation in trades and share market

False and unethical pharmaceutical data integrity practises have resulted in significant regulatory and financial ramifications for an extensive number of companies.
Major issues for why we are dealing with data integrity are:

1. Senior management who doesn't themselves learn and don't support data integrity. They don't promote support or guide the employees for following correct procedures.
2. Employees who lack technical and regulatory knowledge of process or product hence cannot perform their jobs adequately and accurately.
3. People don't understand importance of cybersecurity and data completeness[8]

The U.S. Food and Drug Administration's (FDA) 21 CFR Part 11 was established for the main objective that data integrity is essential to regulatory compliance. Since the FDA launched its first guideline in 1963, it has collaborated with the European Union to develop numerous guidelines on a variety of topics related to data integrity for pharmaceutical companies. Data integrity is security of data from unintentional changes to information protecting data from unauthorised parties.[9]

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Integrity stems from the Latin word 'integer' which means whole and complete. So, integrity requires an inner sense of 'wholeness' and 'consistency' of character.Integrity is not possible without compassion and makes it clear that doing the right thing includes doing it for the right reason[10]

Integrity, ethically can be defined as "the honesty and truthfulness or accuracy of one's actions and a concept of consistency of actions, values, methods, measures, principles, expectations, and outcomes.[11]

Data can be defined as attributes or details, usually numeric, collected as a result of various observations. Data in more technical sense may be defined as collection of qualitative or quantitative variables about one or more persons or objects. In complete sense data integrity is characteristics or information which is complete, accurate and reliable.
"Integrity is doing wright thing when no one is watching"

- C. S. Lewis

II. DEFINITIONS

1. **Meta Data:** Metadata is a contextual data that depends on the preceding or following parts of a text to clarify meaning. In short it is a set of data that describes and gives information about other data. It helps organize electronic resources, provide digital identification, and archive and preserve resources. For example, to denote file size number 23 is meaning less without metadata i.e., the unit KB. Other examples of metadata may be ID given to a person, time or date of activity performed. Many distinct types of metadata exist, including descriptive metadata, structural metadata, administrative metadata, reference metadata and statistical metadata. [12]

2. **Raw Data:** Raw data, also known as primary data, are data (e.g., numbers, instrument readings, figures, etc.) collected from a source. In the context of examinations, the raw data might be described as a raw score. Raw data is primarily unstructured or unformatted repository data. It can be in the form of files, visual images, database records or any other digital data.[13] In a lab when performingexperiment if a provision is made to note the temperature in a reaction mixture every minute and recorded in computer system or record sheet are called raw data.

3. **Static Data:** This document comprises static data and may take the form of an electronic image or a paper record. It is data that does not alter after recording, such as a paper or pdf record that permits little or no interaction between the user and the record matter. For example, once printed or converted to fixed or constant pdfs, chromatographic data lose their ability to be recovered or allow a more complete survey of baselines. [14]

4. **Dynamic Data:** A chromatogram, for example, where the integration parameters (peaks) can be changed, is an example of a data layout that enables the recording of essential relationships between the user and the content recorded. Additionally, it enables the user to modify formulas or entries in record sheets used to compute analytical results or other data, such as computed yield. [1, 9,14]

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5. **Electronic Data:** Digitalization for raw materials maintenance can take many shapes from online certificates of analysis (CoAs) to electronic batch records (EBR). Companies in the pharma industry are now approaching towards more digitalization at various stages of manufacturing. Automated systems benefit us by minimizing the double verification which is mandatory for manual papers. Electronic data includes data from ERP software for controlling lab scale data, quality systems data warehousing data and other records maintained in pharmaceutical industry.[14, 25]

6. **Audit Trail:** It a system that traces the detailed transactions relating to any item in an accounting. record. It is secure, computer-generated, time-stamped electronic recordof the changes that have been made to a database or file. An Audit trail is source of progressive, sequential, consecutive set of records that furnishes secured documentary evidences for series of activities that may have altered or modified at any time of specific procedure, test, operation or event. E.g.,For system-based record: In generation and issuance of Batch manufacturing record from software audit trail will include the record of date and time, BMR number, which regulated market it belongs to, Batch size, product name etc. For a paper record, the initials of the person making the change, the date of the change, and the reason for the change—all information necessary to authenticate and ratify the change—would be documented with a single-line cross-out, preserving the legibility of the original entry. [1, 14]

7. **Backup:** In the event of a system crash or disc corruption, backup data is required. A backup is a set of duplicate electronic files that are kept as insurance in case the original data or system is lost, corrupted, or otherwise unrecoverable. It's crucial to remember that these backup files serve their purpose in storage temporarily and shouldn't be used as an archive system. [1, 14]

8. **Computerized System:** A computerized system consists of hardware, software, operating system software, and supporting documentation. Examples include automated laboratory systems, operating instructions, manuals, control systems, clinical, manufacturing or compliance monitoring database systems. One or more automated processes and/or functions are collectively controlled by a computerised system. [14]

9. **Corrective and Preventive Action (CAPA):** The word CAPA refers to actions done to eliminate or reduce nonconformities or other objectionable, disagreeable, unacceptable, unsuitable, undesirable, or out of place situations in an organisation. CAPA is a widely used concept in GxPs (good laboratory practises, good clinical practises, and good manufacturing practises), as well as a number of ISO (International Organisation for Standardisation) business standards.It is a collection of procedures, laws, or rules that must be followed by an organisation in order to account for duplicate non-conformance in production, documentation, analytical methods, or computing systems. Nonconformity can happen when a production process causes a drop in quality and is not immediately corrected. A non-conformance could be a market or consumer complaint, a machine failure, a whole quality management system failure, or a misinterpretation of written job instructions. Corrective action refers to a measure taken to get rid of the source of a nonconformity and stop it from happening again. Preventive action is defined as action taken to eliminate the source of a possible nonconformity or other unpleasant circumstance. [14]

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10. **Data Governance:** Data governance refers to a way of retaining data in the format in which it is generated, documented and handled in order to assure completeness, consistency, and accuracy throughout the data life cycle. [14]

11. **Data Life Cycle:** The data life cycle is the series of eventsthat a specific unit of data goes through, from beginning of its generation or manifestation to its ultimate recording, documentation and/or deletion, withdrawal and clearance.Data life cycle management inculcates all the aspects of data generation, collection, processing, storing, retrieval, analysing, visualizing, interpretation and its archivalThere should be a structured approach for analysing, monitoring, and managing data so that the risks associated with data may be managed in a way that corresponds to its potential impact on product quality and patient safety throughout all phases of the data life cycle. The data life cycle acts as a navigational tool to assist users in finding suggestions on how to efficiently deal with their data across all stages of the data life cycle. [14]

12. **Good Documentation Practices:** Good documentation is described as standards by which data is collected and framed into document. It can be defined as measures taken safe guard the data collected whether on paper or digitalized system continue to exist as traceable, legible, attributable, intact, permanent, original and accurate throughout the document longevity. [14]

13. **GxP:** A collection of rules and standards known by the abbreviation GxP were developed to guarantee the security of products used in the life sciences while upholding the standard of operations at every stage of manufacture, control, storage, and distribution. GxP is a shared term for guidelines and regulations under good practices which ensures the quality in many domains of globally recognised paradigm such as GMP (Good Manufacturing Practice), GCP (Good Clinical Practices), GLP (Good Laboratory Practice), GDP (Good Documentation Practice), GSP (Good Storage Practice), GDP (Good Distribution Practice) and GRP (Good Review Practice)[14]

14. **Hybrid Approach:** Hybrid approach is a mix up computerized system where combination of original paper records andelectronic records that combine to give information about the complete data set which is then reviewed and maintained. E.g., while doing analysis the analysts, with generated electronic record even takes a print of the result for summarizing the total data. When hybrid approach is used its mandatory to use appropriate controls for recording data like templates, master documents, recording sheets according to SOP.The link between the original record and paper record should be such that they be legible throughout data life cycle.[16]

15. **Master Controlled Documents:** Mater controlled documents are approved documents which are in custody of QA department and can be issued for operational work after doing requisite issuance formalities. Examples of such documents are master SOP, Validation Protocols, Manufacturing\Production Records, master packing recordQC specifications etc.[16]

16. **Original:** Original records are the preferable versions of records and refer to the first generation of data. Records should be archived to the greatest extent practicable for an original record.[16]

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17. **Post-Dating:** Post-dating means assign a date later than the actual one to (Enter a data before activity is performed).[16]

18. **Back- Dating:** It means is executing a document and then dating it with an earlier date than the actual date of execution.[16]

19. **True Copy:** A true copy is a copy made from the original record. It is a certified document showing all the details of the original documents but is not original document. It confirms that the it contains the same data or exact data mirror imaging to original data.[16]

Pharmaceutical industry is the vital segment of health care system as it deals with manufacturing of medication for patients intended for safe and therapeutically active with good consistent quality. The creation, certification, registration, commercialization, and life-cycle governance of pharmaceutical products all depend on the integrity of data management, which is a critical component of good manufacturing practises (GMP). The GDPs help us avoid collecting inaccurate data when making and analysing pharmaceutical products. Product quality and patient safety would be affected, either directly or indirectly, by this. GDP compliance is required by both the US and European regulatory agencies, the EMA (European Medicines Agency) and the USFDA (United States Food and Drug Administration). A number of international organisations, including the World Health Organisation (WHO), Health Canada, and EudraLex (European union collection of standards for fundamental legislation governing medical products), together with the United States Pharmacopoeia (USP), have released certain guidelines corresponding to GDPs.Not only the regulatory requirements but maintenance of authentic records is also important as documentations of activities in the pharmaceutical industry allows critical evaluation of internal procedures and continuous improvement of process and minimizes the time for repetitive detailed study from start to end.[17]

The WHO states that the goal of good documentation practises is to:

- Set a predefined specifications and operational strategies for all materials and processes of manufacturing and analysis,
- Set job responsibilities of employees based on their expertise field
- Ensures release of a product to be done after all the necessary information to authorized persons
- Maintain documented evidence for future investigations and audits in a legible manner
- Maintain constant availability of data for statistical and validation analysis.

For an organisation to be compliant with regulatory authorities and to enhance market value it is important to:

- Provide requisite resources for fulfilling complete documentation,
- Ensure that the documents are prepared with concept of ALCOA+. (Attributable, Legible, Contemporaneous, Original, Accurate, Complete, Consistent, Enduring and Available)

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- Fill the gap of any missing data for existing documents providing basis of forecasting what is to be done in future
- Continuous training should be provided to employees on existing and current GDPs to be followed in manufacturing ang quality control departments.

Quality Assurance department needs to review all the documents before release of product to market [17].

III. TYPES OF DOCUMENTATION IN PHARMACEUTICAL INDUSTRY[18]

Both printed electronic forms and electronic systems are considered documentation..

1. Standard Operating Procedures(SOP)
2. Records/Worksheets
3. Annexures
4. Quality management system documents
5. Production and packaging instructions
6. Standard operating procedures
7. Records.
8. Licencing documents
9. Master documents
10. Technical agreements
11. Confidentiality agreements
12. Training records
13. Qualification Documents(URS, DQ, FAT, OQ, PQ, IQ)
14. Quality Manual
15. Issuance Documents
16. Annual product quality review (APQR)documents
17. BMR/BPR
18. Validation protocols and reports
19. Deviation reports
20. Audit plans
21. Validation Master Plans and validation documents
22. Test material related documents including test material receipt, product specification, and reports
23. Documents pertaining to personnel, such as training records
24. Documents related to facilitywhich includes floor plans, HVAC plans, and environmental specifications
25. Planned Deviation, unplanned deviations and system failure investigation
26. Change control
27. Logbooks, worksheets, and notebooks

In pharmaceutical industry it is said that "**If It Is Documented It Is Done**". So, recording and maintenance of data plays a very important part in pharmaceutical industry.

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IV. CONCEPT OF ALCOA TO ALCOA+

For prevention of data integrity issues concept of ALCOA was utilized by pharmaceutical companies. ALCOA is concept to implement for the data guarding and sturdiness in pharmaceutical industries. In 1990's Stan Woollen used the acronym ALCOA when he worked for the agency to help him remember compliance terms relevant to data quality which has been widely associated with data integrity by FDA. [19]ALCOA defines a framework to achieve and maintain data integrity, especially important for ensuring good manufacturing practices in regulated industries. The theory of ALCOA can aid to furnish an audit trail data that encapsulates details such as additions, deletions, or alterations of data in an electronic record without obscuring the original record. [20]As an improvement over this, additionally, new characteristics were identified which are critical to data integrity apart from regular good documentation practices core characteristics. This launched the improved ALCOA now known as ALCOA (+).[21]The term ALCOA which is acronym stands for Attributable, Legible, Contemporaneous, Original and Accurate. Later ALCOA was outstretched to ALCOA-C or ALCOA+ (Fig 1). Complete, Consistent, Enduring and Available (CCEA) was added to ALCOA in 2010. They are considered as the heart of Good Documentation Practices because of the specific significance they hold. [22]

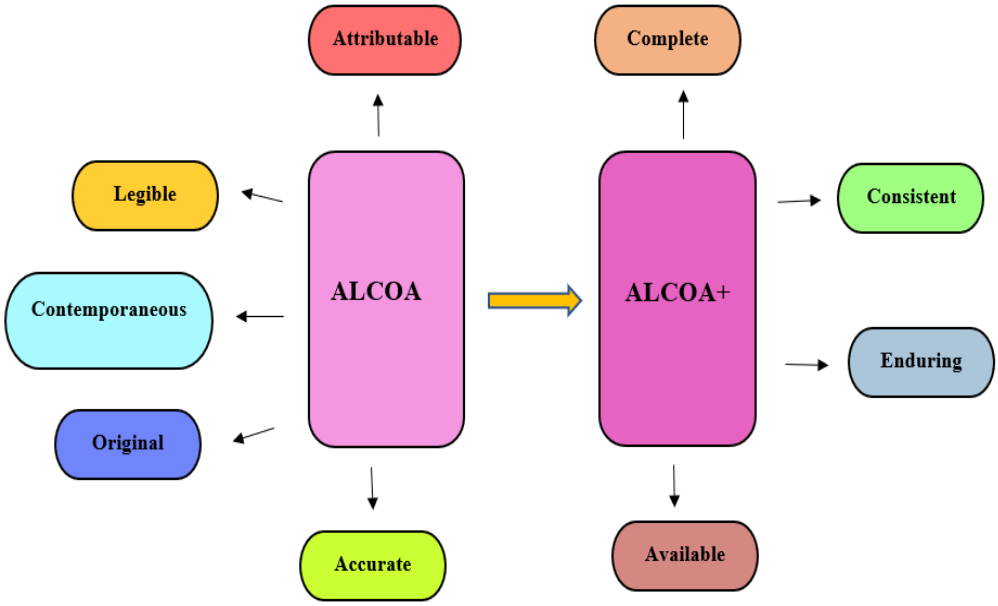

Figure 1: Outstretching to ALCOA to ALCOA+

Following are the features for Quality records considering Good Documentation Practices in place.

1. **Attributable:** When and Who performed an activity? It indicates that the any data should be traceable and determinable regard of when it is performed and who performed it. It should be capable to discover and find out the emergence and the past of data. The record should also be able to identify the person who initiated, collected, preserved, or managed the information. This produces trace-ability in urgent situations. Consequently, accountable is sometimes regarded as traceable. [[22, 23,24]

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Example: System user ID sharing and password sharing, Analysis done by two person and one person signing

2. **Legible:** Data should be readable throughout its entire life cycle? Data should be readable throughout its entire cycle. The document should be simple to read, accurate, and intelligible, in other words, relatable and readable. This is necessary because data must be readable and understandable for years or even decades after it has been captured. History for the particular data should be relevant and understandable even after a gap of long time period.[19, 25]
   Example: Hand writing should be readable by others, Write over's- usage of pencils or erasers/ correction fluids

3. **Contemporaneous:** Is the documentedat the time of the activity? Contemporaneous means Parallel, Simultaneous, or Concurrent. The data should be recorded at the time when activity is performed. Data should be recorded at the same time they are generated, collected and observed(include time and date stamps for electronic records). Data should never be backdated, or forms completed with expected results prior to execution.[26, 30]
   Example: Data should be recorded at the time of activity, Back dating or forward dating (Non-compliance with GDP)

4. **Original:** Is the information gathered original or certified copy? The original word refers to the availability or existence of raw data, whether on paper or electronic data. Sometimes the prints which are generated electronically or digitally fade away over a period of time, in those cases the document should be scanned or photocopied and saved for future references.Original data refers to the paper or electronic media on which the data point was first recorded, such as logbooks, protocol, registers,notepad, worksheets, data archives, or software tools. It is best to get in the habit of entering the data directly into the main register or original notebooks rather than writing it down on a piece of paper and finishing it later. This could lead to more errors and impair the ability to authenticate original data. Original data is defined as data or information that is documented at the time of data generation and includes all subsequent data required to assure the quality and proper conduct of GxP activities. A second person verifies a certified copy by comparing it to the original, ensuring that it is exact, correct, and complete and maintaining the original content and importance in its original form. [27, 30]
   Example: Altering/ modifying and deleting original data, Analytical results written on new worksheet as original data got impaired (smudged or torn off or discarded)

5. **Accurate:** Are there any errors or editing without documented amendments? Consistent, factual, error free and recorded as it is. For validity of data, it should be error free. In case of amendments there should be evidence for accompanying support for changes made. The quality of data should be maintained such that any changes made during any time of data life cycle has enough proof for supporting the change made. If any editing is done it should be done considering GDP.[26, 27]
   Example: System adjusted to get passing results to avoid OOS (out of specification), Data from passing run analysis is used for another sample to expect a result within specification

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6. **Complete:** Is all data documented viz. any test, repeat or re-analysis performed? Data for any repeat or reanalysis performed on the sample should be noted correctly.Evidences of the data for any reanalysis performed should not be deleted(Evidence: Audit Trail). Evidence should be such that, when reconstructing or reforming the events, data must be sufficient and complete for required information. This means that there should not be any breach when remodelling of requisite information. All paper and electronic data, including all tests (original and retest), must be properly recorded, clearly identifying when and who performed the test, and ensuring that nothing is missing.[25, 30]
   Example: Information worksheets without name of person, instrument ID, date and time etc, meta data support is not mentioned for the original raw data collected

7. **Consistent:** Whether all the components of the investigations are always carried out similarly? Consistent with reference to the events in chronological order.Records should be maintained in such a way that data should reproduce correct information consistently at any time. Any information that will be entered into a database must be valid and adhere to all the established guidelines for that system. Data consistency does not guarantee that the transaction is accurate in every way. The main purpose of it is to find any programme faults that caused the violation. [21, 23]Data should be collected with the use of a system that enforces the use of approved data gathering and analysis methodologies, reporting templates, and laboratory workflows.
   Example: Batch records not filled on time by the operators, Flash results which tend to disappear before analysts can jot down the result.

8. **Enduring:** Is all the datarecorded systematically in laboratory notebooks or in validated systems? The data or information must be maintained in such a way that it should remain unviolated, pristine, approachable and accessible throughout their specified retention period. Paper or electronic data should be transcribed in validated software systems and defined laboratory notebooks, registers, log books, spreadsheets, worksheets, annexures etc. Data should not be recorded in any scrap papers.[19]
   Example: Tampered batch records, deletion or modification of existing system data due to upgradation of system.

9. **Available:** Can the data be accessed for review, audit or inspection over the lifetime of the record? Availability of the data severs the basic purpose of saving it so that it can be referred when needed. Data should be readily available when required for inspection and audit purposes. During regular inspection of authorities, the requested data should be produced instantaneously in legible format. Electronic and Paper data should be neatly arranged in a manner so that retrieval and recovery is easy. [19, 30]
   Example: Back up for the files are not available and deleted in case of OOS results, Archival of data is not done until data retention period

V. SIGNIFICANT ISSUES OF DATA INTEGRITY

Majority of the data integrity issues and Form 483 are due failure in quality control instruments.

1. **There are Numerous Causes of Data Integrity Problems. Some of them Include the Following. [28]:**

- No support record for raw data or loss of data during changes made in digitalized system
- Inaccurate and incomplete records or discarding the data of repeated tests, trial runs, sample runs
- Using CoA(Certificate of Analysis) of one batch to release another
- Backdating
- Manipulating integration parameters of chromatogram to get pass results
- Deletion/manipulation of electronic records
- Shutting down audit trail
- Signing instead of concerned person authorized for the work
- Deficient/In complete computer validation.
- Activities not recorded contemporaneously
- Sharing Login IDs by other analysts. By doing this work done by individual analyst cannot be identified.
- Ditto markings are used
- Use of a signature stamp
- Not using ink in the manner prescribed by protocol
- Incorrect ink was used for entries during the spillage, resulting in unintelligible data.
- Failure to identify person who made corrections of changes in logbooks
- Arcane original data
- Usage of pencil
- Erroneous/Fallacious records
- Correct dating of handwritten changes not done
- Multiple line-throughs, write-overs, and the use of "white-out" or another masking technique

The most common GDP violations occur when corrections of the errors are done during recording of information.

2. **Following Methods should be Carried Out for Correction of Documentation (Figure 2):**

- A single line should be drawn across the mistake
- Mention the correction next to the error
- Explain the error,
- Date and sign of the individual carrying out the correction
- Mention time in 24 hr format

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Recording Time	Recording Date	Recorded by	Verified by
~~16:30~~ * 16:32	09th June 2020	NSK 09th June 2020	NSS 09th June 2020
16:35	09th June 2020	NSK 09th June 2020	NSS 09th June 2020
~~16:41~~ # 16:43	09th June 2020	KPS 09th June 2020	NSS 09th June 2020

* ® NSK 09the June 2020

® KPS 09the June 2020

Figure 2: Method for Correction of Document

These common errors should be highlighted in training of good documentation practices. [9, 28] As it is said **"Prevention is better than cure"** industries must follow the Good Documentation Practices actively.

VI. HOW TO DECREASE THE RISK TO DATA INTEGRITY?

1. **All Computer Systems should Comply to 21 CFR Part 11 Guidelines:** The FDA regulation 21 CFR Part 11 is concerned with electronic records. Make sure the electronic data is accurate, comparable, and consistent with paper records. Data must adhere to quality requirements in order to be kept in digital systems.

2. **Software Development Lifecycle Must be Followed:** To supervise the quality task performance, a software development lifecycle methodology can be followed. This methodology aids to investigate different functions of software lifecycle phases which embodies software testing, software development, installation and amalgamation of ongoing system maintenance. All digitalised systems should be suitably qualified, developed, quantified, analysed, investigated, and on routine basis they should be validated.

3. **Validation of Computer Systems:** Software system validation is essential because it gives proof that a certain manufacturing procedure regularly produces products that meet pre-established requirements and quality standards.

4. **Implement Audit Trails:** The date and time of data entries, changes, individuality and deletions made should be recorded by a secure, digitally-generated, time-coined audit trail. Audit trails provides evidence that data entered in electronic records are true, ethical, meets necessary data conversation, and promises that information recorded have not been edited, modified or deleted.

5. **Software for Detecting Errors should be Used:** There must be an incorporation of automated inspection software that will authenticate critical documents which will assure systems meticulousness. Manual examining or inspections are not sufficing to furnish that data are error-free.

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6. **User System Access should be Maintained to Secure Your Records:** All systems containing at least two different pieces of information should have a secured login that only allows concerned parties access in order to ensure data integrity.

7. **Maintenance of Backup and Recovery Procedures are Important:** In case of unexpected data loss or system errors backup and recovery strategy are necessary. This practice guarantees the physical and critical recovery of database ensuring veracious restoration of data.

8. **SOPs and Logical Controls should be Followed to Design a Quality Management System (QMS):** Standard operating procedures and guidelines collectively builds a Quality Management System which lays foundation for ensuring quality into process by systematically organising the process. For ensuring clear liability it is mandatory to follow efficient procedures.

9. **Establishment of a Vendor Management Qualification Program is Necessary:** For ensuring that all the products manufactured are genuine and authentic it is mandatory to examine all the vendors supplying raw materials. Initial validation followed by continuous verification must be carried for ascertaining quality production. Organizations must interact with vendors for new or updated data integrity procedures they follow.

10. **Training of Users and Maintenance of Records Must be Done Appropriately:** For a job to be performed in predetermined manner precise and appropriate training must be given with the help of expertise's. Proof of the training delivered must be documented.

11. **Conduct Internal Audits to Evaluate Procedures:** For a company to thrive in competitive business environment, routine internal audits aids to understand the flaws in regime followed by the firm which empower firms to continuously prosper. Self-inspection by the company under an internal auditor who is trusted consultant assigned for advising upper management on how to best manage the company's quality management system (QMS). [29, 30]

VII. CODE OF FEDERAL REGULATIONS (CFR) AND DATA INTEGRITY [31-36]

With respect to 21 CFR Guidelines includes:

- Part 210 – Current Good Manufacturing Practice in Manufacturing, Processing, Packing, or Holding of Drugs; General.
- Part 211 – Current Good Manufacturing Practice for Finished Pharmaceuticals.
- Guidance for Industry Part 11, Electronic Records; Electronic Signatures — Scope and Application. Table 1 represents data integrity issues related to 21 CFR guidelines in pharmaceutical industry. [23]

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Table 1: 21 CFR Guidelines Associated Data Integrity Issues

Sl. No.	21 CFR Guideline No.	Guidelines	Data Integrity Issues in Pharmaceutical Industry
1	211.192	Failure to adequately investigate any inexplicable disparity or a batch's failure to meet any of its standards, whether or not the batch has already been delivered, or any component of a batch failing to meet any of its specifications	OOS and partial batch rejection were the results of the investigations for a tablet with a specified unit dose (mg) being insufficient. Investigation revealed an OOS for a certain lot's tablet thickness. An evaluation of the production processes and the partially released completed lot that wasn't subjected to stability was left out of the assessment.
2	211.188(b)(11)	Failure to keep track of batch production and control records that contain proof of completion for each important step in the production, processing, packing, or storage of each batch of pharmaceutical product.	Batch Manufacturing Record of the firm lacks to include appropriately statement and control of the processing steps at each stage of manufacturing/ production of products.
3	211.194(a)(8)	Failure to confirm that laboratory records contained complete data generated from all tests necessary to assure adherence to established specifications and standards	In a laboratory for Viscosity testing the quantity samples is critical. Results will be low if sample is too less and results will be high if sample is more. But the firm failed to mention the amount of the sample used for the test.
4	211.68	Failure to implement sufficient controls over computer or connected systems to ensure that only authorised people make modifications to control records and master production, or other records.	For computer or digitalized systems there is not much appropriate controls on its utilization by individuals. The user access controls or password protection with relation to the state of the goods in inventory were not effectively validated by the software meant for drug material and inventory control, drug production scheduling, and control over the distribution of finished products.

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5	211.100(b)	Failure to adhere to written manufacturing and process control methods intended to ensure that the pharmaceuticals you create have the identity, strength, quality, and purity they claim or are represented to have, and to record the same at the time of performance.	Firm fails to follow manufacturing process and online entry control parameters that will define the quality and purity of the product to conform with predetermined specifications.
6	211.160	Failure to follow and record the necessary laboratory control procedures at the time of performance	Any extra tests or procedures that the firm does that are not covered by a client-specific process must be listed in the Supplemental Method Information Sheet (SMIS). A SMIS then becomes a component of the company's official testing procedure. The material should be introduced to the viscometer in little amounts dispersed over the cone and plate rather than all at once, according to laboratory literature on the bulk release testing procedure. In addition, lab SMIS states that the quantity on the sample cup is important for units of measurement.
7	211.165(a)).	Your business does not have a suitable laboratory evaluation of satisfactory compliance to the drug product's final requirements, including the identity and concentration of each active ingredient, prior to release, for each batch of a drug product.	The average of three samples tested for each batch is used to determine potency results in accordance with the sampling technique. The company did not establish an acceptance standard for the standard deviation of test results or the outcomes of individual tests. Therefore, current procedure allowed the release of sterile medicinal products even while individual potency test results were sub- or super-potent in comparison to the potency release criterion of the finished drug product.

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VIII. STATISTICAL TRENDS OF DATA INTEGRITY VIOLATIONS IN PHARMACEUTICAL INDUSTRIES

1. **Statistical Trends of Data Integrity Issues:** The Food and Drug Administration's Bioresearch Monitoring (BIMO) Program's warning and closeout letters were issued between the US fiscal years 2007 and 2018. The letters were analysed by grouping regulatory violations into topics. For the purposes of this study, 300 warning letters in total were examined. All warning letter categories contained instances of failure to follow and maintain processes as well as poor documentation practises. The premarket side of the pharmaceutical sector continues to experience severe data integrity and other regulatory compliance challenges, despite the fact that the number of warning letters has reduced over the past decade and inspection outcomes have been improving. [37].

Table 2and Figure3unveils Data Integrity Associated Warning Letters by different countries for the calendar year 2008 to 2018, along with a cumulative total[38].The pie graph Figure 3 shows that since 2008, the number of nations receiving warning letters about data integrity has increased, with over 80% of those warning letters occurring in the last four calendar years. The warning letter-related sites in 2018 were located in 11 different nations. The number reached its pinnacle in the calendar year 2017, and it will be remarkable to observe if it declines once more in 2019 like it did in 2018. [38].

Table 2: Data Integrity Related Warning Letters by Different Countries

	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	Total
China	1	1	3	1			2	2	14	19	15	**58**
India	1	1		2		6	7	10	9	12	6	**54**
US	1	2	1	1	1				7	15	8	**36**
Europe		1					1	2	6	3	1	**14**
Japan	1								2	1	3	**7**
South Korea										2	4	**6**
Canada			1	1						2	1	**5**
Mexico					2					1	1	**4**
Brazil									3			**3**
Thailand								1				**1**
UAE					1							**1**
Jamaica					1							**1**
Singapore										1		**1**
Australia											1	**1**
Taiwan											1	**1**
Dominican Republic											1	**1**
Total	**4**	**5**	**5**	**4**	**6**	**6**	**10**	**15**	**41**	**56**	**42**	**194**

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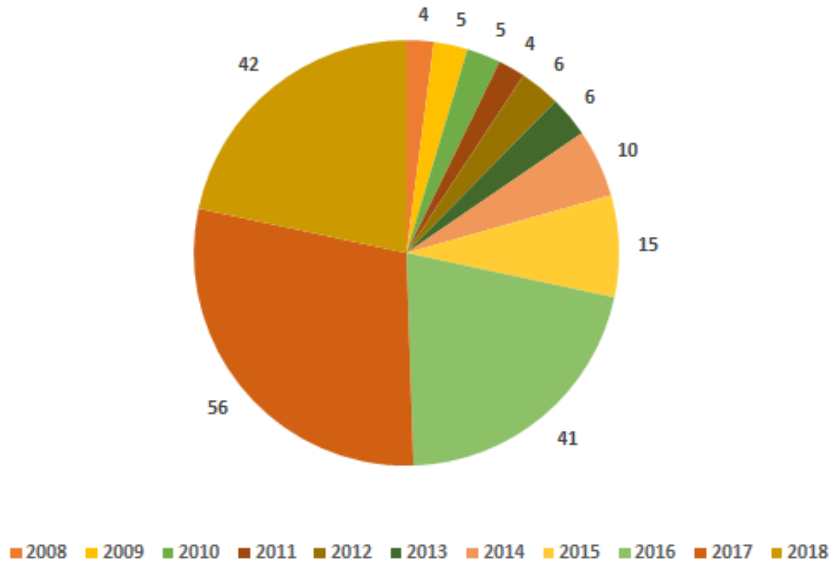

Figure 3: Pie Chart for Data Integrity Related Warning Letters of Different Countries

Table 3displays a clear picture of major of countries involved in data integrity warning letters viz. CHINA, INDIA and UNITED STATES. FDA did not classify numerous violations as "conclusions" or "data integrity remediation" that required a response from the pharmaceutical industry. Warning letters sent to API manufacturers do not mention 21 CFR 211. The FDA agency stated objective of concentrating on the assessment of predicate rule requirements is still followed by the citation of regulations. [38]

Table 3: Data Integrity Related Warning Letters with Reference to Geographical Locations

Country	Total Number, 2008-2018	% of Total, 2008-2018
China	58	30%
India	54	28%
United States	36	19%
Europe	14	7%
Rest of the World	32	16%

Poor documentation practises and failure to follow and maintain processes were the most prevalent violations identified across all warning letter categories Table 4. Approximately 50% of all global drugs 483s that were issued between 2014 and 2018 were reported due to data integrity issues. The Big Data and AI Analytics company Govzilla discovered this information. Data integrity issues were identified in 79% of API warning letters sent out globally during this time. Defects in data integrity have been seen to significantly increase in recent years. [39].

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Table 4: Most Recurrent Data Integrity Related Warning Letters

21 CFR Reference	Frequency of Citations	Caption of CFR Section
211.194	10	Review of entire data, Lab Records
211.188	6	Batch Manufacturing and control records
211.165(a) and (b)	5	Testing and release for distribution
211.192	5	Deviations, Risk managements, Investigations, Production records review
211.68	2	Automatic, Mechanical and Electronic Equipment

For violations of 21 CFR Part 11, very few form 483s and warning letters were issued. The majority of the flaws were attributed to failure to follow 21 CFR Parts 210, 211, and 212's cGMP regulations.

2. Two Major Violations were Cited for CFR Code 211.68 and 211.194.

- Part 211.68 outlines the specifications for "Automatic, Mechanical and Electronic Equipment." Frequently used references in this field include:

  - Mismatch of the printed data with actual audit trail.
  - Data was not legible and correctly backed up for future reference.

- Part 211.194 is referenced when industries failed to study and consider all relevant facts when making lot release decisions. Frequently used citations in this field include:

  - For meeting the predefined acceptance criteria laboratory analysts delete or manipulate data.
  - Identification for out of specification results becomes difficult for investigations since companies fail to review critical data and/or metadata
  - The test results of analysis are either deleted or corrupted by company which do not have sufficient supportive data.

3. Other Most Recent Inadequacies Include:

- Making use of 'pre-injections' of product samples outside of whole sample sets to check whether findings meet acceptance requirements and, if not, deleting/ignore results.
- turning off audit trails to hide results
- Deletions or alterations of results
- Data reduction through the use of integration suppression settings that would probably result in an OOS
- No proper justification for Aborting test runs

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The FDA has promoted the use of "independent" data integrity evaluations as part of the process for resolving detected concerns. Several instances include:

- On August 10th, 2018, Kyowa Hakko Bio Co., Ltd.'s manufacturing facility in Japan received a warning notice that stated the following: “We recommend that a qualified third party with specific expertise in the area where potential breaches were identified should evaluate all data integrity lapses.”
- On March 26, 2019, a warning letter was issued to the Georgia-based manufacturing facility of Winder Laboratories, LLC. Stating the following: “Your quality system does not adequately ensure the accuracy and integrity of data to support the safety, effectiveness, and quality of the drugs you manufacture…. We strongly recommend that you retain a qualified consultant to assist in your remediation.”
- On June 13th, 2019, Akorn Inc.'s Illinois manufacturing site received a warning notice that stated: “Your quality system does not adequately ensure the accuracy and integrity of data to support the safety, effectiveness, and quality of the drugs you manufacture…. We acknowledge that you are using a consultant to audit your operation and assist in meeting FDA requirements.”

4. Benefits Data Integrity Assessment:

- Builds up focus of Organization on Data Integrity issues– Braces the fact that all employees are focused on compliance of data integrity concerns.
- Satisfaction of identifying data integrity concerns – Once the data integrity issues have been identified it becomes easy to work on compliance.
- Expenditure reductions and Time saving – Data integrity issues identified and rectified internally are much more significant than identified by regulatory authorities and are also less time consuming for redrafting.
- Can concentrate on enhancing Business – Can stay focused on well-being of business in effective way rather than spending time and expenditure on data integrity issues if solved proactively.[39]

Regulatory organisations like the WHO and FDA have published guidance on appropriate documentation practises and how data should be collected and handled as a result of an increase in data integrity breaches during 2019. Draft document of guideline on data integritywas published by WHO in October 2019.

IX. HIGHLIGHTS OF WHO GUIDELINE ON DATA INTEGRITY[40]

1. Guideline covers basic concepts, information and recommendations for promoting GxP in documentation and record keeping which will facilitate compliance with Data Integrity.
2. This guideline does not cover Data integrity on medical devices, however designated as ‘GxP’.
3. this guideline should be studied in conjunction with WHO GxP guidelines and publication, given that it has been harmonised with other published materials.
4. In order to identify and evaluate risk areas, data integrity risk assessment should be carried out in accordance with current GMP practises that provide a risk-based approach over the life cycle of data.

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5. Both contract givers and contract acceptors must abide by this rule. Since contract acceptors are the ones who give them the data, it is the contract giver's responsibility to guarantee that the contract acceptor conforms with the principles emphasised in this guidance.
6. The effectiveness of controls can be verified by identification and review of data with risk-based controls.

X. CONCLUSION

In pharmaceutical industry data integrity plays an important role in regard of maintaining quality product because improper practice allows inferior quality of product to reach patients, so it becomes mandatory responsibility of pharmaceutical industry to ensure efficacy, safety and quality of products. Data Integrity is the most common issue with majority of pharmaceutical industries. Concept of ALCOA+ helps maintain data integrity necessary for following Good Manufacturing Practices. Now a day pharmaceutical industryhas started relying on digital and automated systems, hence it becomes necessary to validate them for minimizing errors related to addition or deletion of data. It eliminates the need to check each and every step of the manufacturing and distribution of pharmaceuticals..Following the ALCOA+ principles is the best way to achieve this goal. Numerous regulatory bodies suggest of using ALCOA as tool to reduce errors and ensure GDP. Warning letters and closeout letters issued by the Food and Drug Administration's Bioresearch Monitoring (BIMO) Program, Big Data and AI Analytics firm Govzilla and article by Barbara Unger suggested most of the form 483 citations were due to data integrity issue. An inflation in multiple numbers of data integrity related violations during recent years roused a vital requisite to publish guidelines on good documentation practices by regulatory bodies.

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XII. ABBREVIATIONS

1 FDA- Food and Drug Administration
2 cGMP- Current Good Manufacturing Practices
3 MHRA- Medicines and Healthcare Products Regulatory Agency
4 WHO- World Health Organisation
5 PIC/S- Pharmaceutical Inspection Convention and Pharmaceutical Inspection Co-operation Scheme
6 GDP- Good Document Practices
7 CFR- Code of Federal Regulations
8 KB- Kilobyte
9 ID- Identity
10 ERP- Enterprise Resource Planning
11 BMR- Batch Manufacturing Record

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12 GxP- Good Practices. (x-Variables)
13 SOP- Standard Operating Procedures
14 OOS- Out of Specification
15 URS-User Requirement Specification
16 DQ- Design Qualification
17 OQ- Operational Qualification
18 PQ- Performance Qualification
19 IQ- Installation Qualification
20 FAT-Factory Acceptance Testing

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Intellectual Property Rights, Naturally Derived Bioactive Compounds, and Resource Conservation

Raju Wadekar, Subhash C. Mandal, and Kalpana Patil

Abstract

On October 20 to 22, 1994, an interim annual meeting was hosted by the American Society of Pharmacognosy in San Jose, Costa Rica. During the symposium, subject experts from all over the nation shared their insights on issues adjoining intellectual property rights in kith and kin of drug development from naturally derived compounds, plant conservation, and international conventions on bioprospecting. To guarantee that the genomic assets of all nations will be developed for the benefit of human race and observant of recent current stratagems for the benefits, scientific collaboration will be warranted. The core issue of the conferences, the fair and justifiable sharing of the compensations of bioprospecting, was articulated from a variety of opposing standpoints, as can be seen from the summaries of presentations. These divergent belvederes are the consequence of various explanations. Different viewpoints to the larger society are formed by the multiplicity midst the world, particularly those in developing nations. Additionally, a lot of the discussion and negotiations around access to resources wherever they are discovered are clouded and perplexed by the excessive emphasis on the unearthing, expansion, and business of medications as the inimitable outcome of the exploitation of genetic resources. It should be underlined that academic and research institutions would fail, due to a successful outcome from bioprospecting product. Instead, it is critical to understand that other vital goals may also be achieved, particularly in the fields of technology transfer and capacity building. The symposiums help advance research on intellectual property rights (IPR), naturally occurring bioactive substances, and resource conservation for the moral use of conventional medicines, as well as for the development of a country's economy and healthcare system. In light of the foregoing information, an effort has been made in this chapter to disseminate recent scientific advancement and to comprehend the interrelationship between IPR, bioactive of natural origin, and conservation of resources in order to establish a smooth avenue for traditional drugs and medicine thereof.

Keywords

Intellectual property rights · Bioprospecting · Genomic · Conservation · Bioactive

Abbreviations

BMBs	Bioactive-made biologics
CDSCO	The Central Drugs Standards Control Organization
EGCG	Epigallocatechin gallate
GAP	Good Agricultural Practices
IMS	Indian Medical Systems
IPR	Intellectual property rights
NGOs	Nongovernmental organizations
PPD	Phytopharmaceutical drugs
TEK	Traditional ecological knowledge
TRIPS	Trade-Related Intellectual Property Rights
WIPO	World Intellectual Property Organization

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1 Introduction

According to one definition of traditionally derived medicine, it is "the sum and set of the understanding, expertise, and practices based on the principles, beliefs, and proficiencies native to diverse cultures, whether justifiable or not, used in the preservation of health as well as in the prevention, diagnosis, improvement, or treatment of physical and mental illnesses" [1]. The term "traditional ecological knowledge (TEK)" also applies to traditional communities. As a whole, traditional knowledge (TEK) is a component of ecology science and is crucial to the development of traditional medicine and healthcare systems [2]. In contrast to the Western systems, TEK in traditional societies is carried out by "collective memory" [3]. The amount of knowledge available to humankind about the universe he lives in has increased exponentially. The scientific revolution had begun during the Renaissance and had grown throughout time as information was amassed at an ever-increasing rate. The twentieth century was an era of great change in many facets of life, mostly brought on by advances in science and technology. Although each discovery builds on the one before it, the "individual innovation" is the primary driver of this Western-originated knowledge accumulation. The society gave being "inventive" a lot of support, and it eventually developed ways to defend itself. The primary system for safeguarding innovation is a "patent." The world receives thousands of new patent applications each year. In the history of scientific creation, inventors have on occasion decided to reveal their discoveries without any financial incentive or with the hope of profiting from patents. In the current international economy, patenting is advantageous, and it positively influences invention. However, as the scope and accessibility of the global economy expand to every corner and area of the earth, some new issues surface that are difficult to address using the traditional private rights approach. A type of patenting in the area of intellectual invention is known as an intellectual property right (IPR). Intellectual property also encompasses copyright, trade names, and industrial designs, but, in this article, patents are solely discussed in relation to conventional medicine. The outcomes of Western science are essentially recalled when innovations are discussed, but there are also genuine inventions that are protected by IPR since they were the fruit of thousands of years of knowledge accumulation based on the experiences of traditional societies. One of the areas of research that has generated the most interest and questions concerning the locals' intellectual property rights (IPR) is the treatment of diseases that are unknown to contemporary medicine. A number of perspectives on the problem are presented by the conceptual framework on the domains and relationships of property and related rights [4–6]. Biopiracy, which is the act of using information without providing any profit to the owner of the entity or information, poses a concern for the future but is already a reality today and carries with it a number of legal repercussions [7]. IPR has evolved into a metaphor for indigenous ownership of all traditional knowledge, opening up possibilities for contractual procedures to guarantee benefits go back to the original cultures and nations. The IPR discussions shed light on how important biodiversity is to human health. The basis of pharmacy has been plants from antiquity to the present, but, as time goes on, the pace of extinction of species and cultures increases, and the health of humans continues to decline due to diseases for which there is now no solution. As a result, the significance of plants as medicines is now highly acknowledged, and the IPR related to their usage has been contested globally [8]. Also, efforts have also been made to utilize naturally occurring bioactive substances as delivery agents, on the other hand. Such bioactive, naturally occurring compound-derived nanocarriers, nanovesicles, phytosomes, microemulsions, etc., are anticipated to display intrinsic therapeutic efficacy in addition to having greater biocompatibility than drug delivery systems produced from synthetic materials. The active components of green tea, epigallocatechin gallate (EGCG), curcumin, and resveratrol, are typical naturally occurring bioactive chemicals that have been employed as carriers in biomedical applications. The ability of these delivery carriers to treat diseases is illustrated by a number of examples, and their potential for biological applications is evaluated [9]. 90% of the above 1400 medicinal plants used in Europe come from wild sources; in the USA, about 120 of the top 165 prescription medications are derived from natural origin [10]. According to a very conservative estimate, in every two-year earth is trailing substantial of each significant drug candidate and the rate of plant extinction is currently between 100 and 1000 times higher than what is expected by natural extinction [11]. Globally, 50,000 plus and 80,000 kinds of flowering plants are used as medicines, as per the International Union for Conservation of Nature and the World Wildlife Fund. Among them, 15,000 species are in danger of going extinct due to habitat loss and overfishing [12], and 20% of their wild resources have already been almost completely depleted due to the growing human population and plant consumption [13]. The risk of extinction of medicinal plants has increased globally, despite the fact that this apprehension has been known for years, particularly in China [14], India [15], Kenya [15], Nepal [15], Tanzania [16], and Uganda [16]. Numerous studies have been conducted on the preservation and sustainable usage of medicinal plants [17, 18]. A number of sets of recommendations have been made about their conservation, including the necessity for integrated conservation approaches based on both in situ and ex situ tactics [19] and the construction of

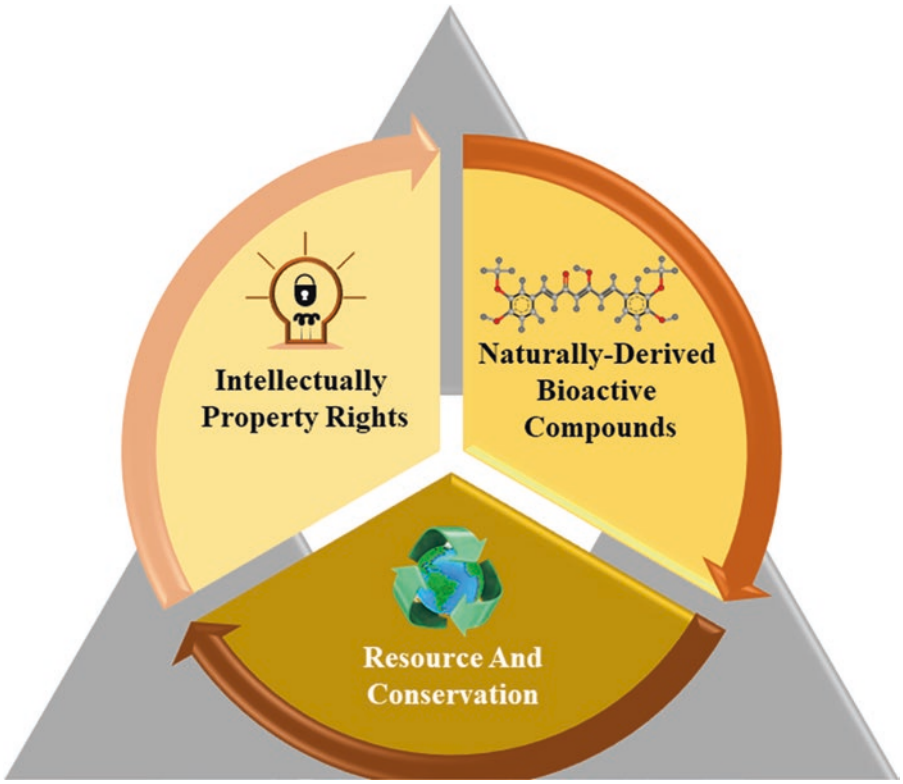

Fig. 1 Golden triangle and interrelationship between IPR for traditional knowledge of medicines/drugs

systems for species inventorying and status monitoring. Sustainable use of wild resources can be a successful conservation solution for medicinal plants with diminishing stocks. Due to the enormous needs of sizable populations, the situation is particularly dire in China and South Africa. Therefore, it is warranted to understand and apprehend the recent knowledge regarding the appropriate use of the golden triangle (Fig. 1) and interrelationship between IPR for traditional knowledge of medicines/drugs, naturally occurring bioactive compounds, and its exploitation by modern science in drug delivery and its resource and conservation in nation economy building and to provide better healthcare system to the world.

2 Intellectually Property Rights (IPR)

IPR is a weapon used by the law to safeguard industrial innovation in the contemporary era, as well as to encourage biodiversity preservation and ensure just and equitable benefit distribution among indigenous custodians [20, 21]. The majority of patented inventions are built on existing information. One benefit of the patent system is that it encourages new ideas by utilizing publicly available knowledge on the basis of nation-to-nation cultural heritage and beliefs [22]. Patent laws protect many herbal products that are derived from conventional medicine. In terms of copyrights, patents, trademarks for pharmaceutical preparations, and registered designs, phytochemicals have a wide range of intellectual property rights (IPR) [23]. Trade secrets and trademarks are the two most common types of IP protection for herbal medicines [24]. However, patenting plant-derived pharmaceutical goods and methods based on conventional wisdom has grown to be a significant intellectual property rights issue [25]. Indigenous knowledge and intellectual property rights have resulted in numerous difficult legal issues for the modern world. Indigenous knowledge preservation concerns extend beyond the realm of the law. Furthermore, communities all across the world can directly experience the issues raised by intellectual property legislation in traditional knowledge systems [26]. The word "biopiracy" is now frequently used by activists and some specialized nongovernmental organizations (NGOs) to refer to traditional knowledge regarding the incorrect or illegal usage of biological materials [27]. Additionally, the rising patent count reflects increased research into biopiracy. Indian neem tree cases have demonstrated the crucial role patents play in biopiracy. These international consultants are hesitant about the overuse of the term "biopiracy" to define particular instances of unfair claims over biological resources and traditional knowledge, despite the fact that there has been much international discussion on disputes pertaining to intellectual property and assets, traditional knowledge, and heritage. The word "bioprospecting" is rela-

tively new and was created to define the responsible use of natural resources, respect for indigenous peoples' rights, and identification and commercialization of bio-products [22, 27–29]. The rights and interests of the traditional knowledge owners who give the "source" of these patents (such as disclosure of sources and profit sharing) must also be honored while we preserve these leading compounds and secure private rights under the current patent law system. Otherwise, it violates fundamental intellectual property system principles and ideas like the interest balance [30, 31]. A significant agreement that supports the harmonization of intellectual property rights (TRIPS) [32]. New contexts for the protection of ethnic interests have been reputable with the help of imperative organizations like the World Intellectual Property Organization (WIPO). In addition, nations and regions are mostly concerned with safeguarding traditional resources [33].

2.1 Traditional Knowledge and Intellectual Property Rights

Traditional agricultural, medicines, and ecological knowledge (TAK, TMK, and TEK) are three types of "traditional knowledge." Native American tribes, peoples, and countries have and employ indigenous knowledge, which is a subset of the outmoded knowledge category. Traditional medical knowledge (TMK) is used by indigenous people, particularly in rural communities, to preserve their healthcare systems [34, 35]. The preservation of traditional knowledge (TK), which is fundamental to the identity of most local communities, is crucial for the social and physical environment of those communities. They have a connection to the local biodiversity, which includes endemic plants, animals, fungus, and other biological materials. A large range of medical plants, healthful herbal remedies, and agricultural and forestry products that are sold internationally and have significant economic worth were discovered, developed, and preserved by traditional tribes and communities. TK thus contributes significantly to the world economy. Pharmaceutical companies have expressed interest in creating traditional medicines from a variety of plant species, which can serve as vital starting points for the development of novel pharmaceuticals. As an example, the indigenous Madagascar *Catharanthus roseus* (Apocynaceae) plant was used to create the anticancer medications vincristine and vinblastine. Patent, trademark, geographical indication, trade secret, or confidential information protection may be available for innovations based on TK. Traditional intellectual property (IP) regimes, on the other hand, do not protect traditional knowledge, which has ancient heritages and is frequently transmitted from person to person orally. By altering well-known qualities, biological resources and/or traditional knowledge (TK) have been claimed as intellectual property (IP) rights [36–38]. However, there are some concerns that need also be addressed with regard to preserving traditional knowledge and cultural manifestations [34, 35]. It is disturbing to learn that researchers from universities and major corporations have a keen interest in aboriginal plant use today for the purpose of obtaining patents to profit financially, putting the lives of millions of people living in tribal communities around the globe in danger [34, 35, 39]. We must reverence the rights and interests of owners of traditional knowledge who provide a "source" for these patents while using the current patent law framework to protect these prominent amalgams and safeguard private rights. Additionally, the possibility of patenting traditional medicine-derived products offers a significant encouragement for pharmaceutical syndicates because it produces potential profits that could accrue over-time ,and, eventually, partaking in such information would value humanity [31, 40]. Attempts to patent indigenous plant knowledge in some areas where people have been using it for centuries have frequently prompted harsh criticism. Patents issued in relation to the neem tree are an example of this. By revoking a contentious patent on the antifungal medicines derived from the Azadirachta (neem) tree, the European Patent Office (EPO) has raised awareness of biopiracy around the world [41, 42]. Traditional knowledge resources may be embezzled if they are used for commercial or industrial purposes, and this can sway the rightful custodians' interests. TK must be protected and nurtured for long-term development in order to serve the interests of those who have traditional knowledge in the face of such risks. For developing nations in particular, it is crucial to preserve, defend, and promote local communities' inventions and practices that are founded on traditional knowledge [43]. As per the summary of IPBES Global Assessment on Biodiversity and Ecosystem Services 2019 for Policymakers: The quality of life for indigenous peoples and local communities and the conservation, restoration, and sustainable use of nature, which is crucial for larger society, are frequently improved by acknowledging their literature, practices, institutions, and values. Legislation and governance rules and regulations can be an effective way to protect nature and its welfare to people by integrating locally adapted management systems and ethnic knowledge. Aboriginal peoples and indigenous communities can benefit from national recognition of land tenure, access, and resource rights in agreement with national legislation, the use of free, prior, and informed consent, amended alliance of benefits resulting from the use, and co-management agreements with local communities [41, 44].

2.2 Protection of Traditional Knowledge

The draft of an international legal instrument created by the World Intellectual Property Organization (WIPO) for the protection of TK licenses to those outside the nation or community of its traditional holders. This effort is being done through the Inter-Governmental Committee on Genetic Resources, traditional knowledge, and Folklore (IGC-GRTF). Traditional knowledge is being sought after for two different types of intellectual property protection [39, 45]. Defensive protection: As an illustration, India created the Traditional Knowledge Digital Library (TKDL), a searchable database that serves as proof that certain treatments are already used in indigenous systems of medicine. This database also works to prevent patents from being granted, which prevents biopiracy, which was made famous when the US Patent and Trademark Office granted a patent for turmeric. Defensive protection is intended to stop infringement and the use of IP in new inventions. A good example of a defensive protection measure is TKDL [38, 39, 45, 46]. It gives communities more power to advance their traditional knowledge, regulate its applications, and profit from its economic exploitation. A handful of nations have also created particular legislation, and some uses of traditional knowledge may be protected by the current intellectual property system [39, 45]. The other paradigms of IPR for the protection of traditional knowledge and resource are summarized in Table 1.

Table 1 Paradigms of IPR for the protection of traditional knowledge and resources

Sr. no	National legislation and policies	Description
01	India's Biological Diversity Act, 2002	India has taken lead in inculcating substantive legislation that is in line with the CBD's goals. It sets rules for the preservation of biological diversity, sustainable usage, and fair distribution of the rewards from the exploitation of biological resources and knowledge [47, 48]. It is forbidden to file a patent in India without first receiving prior approval from the National Biodiversity Authority (NBA) along with the consent of competent authorities for the admission of TK and the completion of IPR applications for goods or inventions using TK [49–51]
02	The Patents Act, 1970	According to the Patents Act of 1970, an innovation is "a new product or a new process for industrial application." The act states that "an invention" is defined in substance as ethnic knowledge. Applications relating to TK and/or biological material must also pass a critical evaluation in accordance with sect. 10(4)(a) and (b) of the Patents Act requirements for full and particular disclosure of the invention [50]
03	Protection of Plant Variety and Farmers' Rights Act, 2001	The Ministry of Agriculture is responsible for administering India's unique legislation, the Protection of Plant Variations and Farmers' Rights Act, which was passed in 2001 and protects farmers' rights and plant variations. The act establishes a framework to safeguard plant varieties and farmers' and plant breeders' rights, particularly those relating to their assistance to the accessibility and preservation of plant genetic resources for new plant varieties [52, 53]
04	The National Green Tribunal Act, 2010 (NGT Act, 2010)	For the instant tenacity of civil matters involving environmental protection, forest conservation, and other natural resource preservation. The tribunal contributes significantly to environmental development that is sustainable [53]
05	Forest Rights Act, 2006	The act protects individual rights to the community to owe the cultivation process, other husbandry processes such as grazing, fishing, and access to waterbodies in woods, habitat moralities for predominantly susceptible tribal groups, traditional seasonal resource access for pastoral and itinerant communities, access to biodiversity, community privileges to intellectual property and TK, acknowledgment of traditional accustomed rights, and the right to shield, reinforce, or conserve or maintain natural resources [52, 54]
06	Geographical Indication of Goods (Registration and Protection) Act (1999)	The act was passed by India as part of its compliance with TRIPS obligations. According to the act, a "geographical indication" is a signal that places agronomic, natural, or factory-made goods as coming from or being made in a country's terrain, or a particular province or locality within that region, where a particular superiority, repute, or other distinctive of those goods is primarily attributable to that locality, as well as in the case of engineering. Geographical indications in India include tea from Darjeeling, silk from Kanchipuram, and sandalwood oil from Mysore [55, 56]
07	Traditional Knowledge Digital Library (TKDL)	In addition to developing a databank on the plant's intricate and their therapeutic applications, tangible steps have been taken to build a program aimed at documenting the knowledge and evidence found in the ancient books of ASU. More than 2.90 million Ayurveda, Unani, and Siddha medical formulas have been translated by TKDL into five internationally renowned. A communal tool for managing intellectual property rights is TKDL [57, 58]
08	International Forums: The Nagoya Protocol	It is a brand-new international agreement that strengthens and aids in the application of the CBD, particularly one of its three goals: the equitable and fair distribution of gains from the use of genetic resources. A significant accord in the regulation of biodiversity on a global scale, the Nagoya Protocol, is important for a number of business and non-commercial sectors engaged in the use and exchange of genetic resources [59]
09	Convention on Biological Diversity (CBD)	International agreement was created to embolden the conservation and fair and equal dissemination of the welfare associated with the use of genetic resources as well as the sustainable development of biological diversity. It is aimed at upshot in a justifiable future. The Convention on Biological Diversity protects inherited properties, classes, and environments at all levels of biodiversity [60]

3 Naturally Derived Bioactive Compounds

There has been a tremendous amount of awareness of bioactive substances derived from natural sources, particularly secondary metabolites such as terpenes, flavonoids, alkaloids, coumarins, and stilbenes with anti-inflammatory, immunomodulatory, antioxidant, and other activities. It is crucial to investigate the action mechanisms thereof. It is noteworthy that the impacts of individual biologically active chemicals' antiviral, antibacterial, or other capabilities are more difficult to identify when plant extracts are used. The solute chemicals in extracts may also have complementary or antagonistic effects [61]. At the same time, it is widely understood how plant biodiversity affects the variety of natural chemicals found in plants. Moreover, natural product chemists are ready to avoid continually ascertaining chemicals and identify a wide range of novel secondary plant metabolites [62]. Zhao et al. (2022) identified naturally occurring micro/nanoparticles Naoluoxintong, which are mostly made of carbohydrates, sarsaponins, and proteins and have a typical size of 200 nm. It has been demonstrated that these organic nanoparticles can enhance brain cell viability, lessen oxidative stress, and enhance nerve function. Research on brain protection can be developed using the findings of the study that has been presented [63]. Terpinyl acetate chemotypefrom the genus *Thymus* was examined in the study by Vaiciulyte et al. (2021) to ascertain its phytotoxic and antibacterial properties as a source of natural terpinyl acetate. The findings revealed that just 35% of the *Thymus pulegioides* habitats studied contained terpinyl acetate, a chemical that is extremely rare in essential oils. On fungi and dermatophytes, terpinyl acetate essential oil had a strong antibacterial impact, but not as much on bacteria and Candida yeasts [64]. It was discovered that specimens found near the southernmost point of the species' geographic range in Europe have different chemical properties and antioxidant activity. The concentration of secondary metabolites and the total phenolic contents varied widely measured extract of bearberry plants that grew in two diverse habitat types, heathlands and pine woods [65]. In addition to arbutin, the enriched source of polyphenolic compounds from bearberry is proven for its antioxidant qualities. Bearberry leaves have the potential to be used as a robust natural source of antioxidants in herbal remedies due to their high phenol content and high values for antioxidant indices [65]. Fruit and vegetable bioactive components interact to affect each other's antioxidant activity. The interactions between citric acid, quercetin, and caffeine may be affected by the absence of iron during thermal processing [66]. According to the findings of Engelhardt et al. (2021), the presence of minerals, temperature, and interactions with other molecules can all affect a compound's ability to behave as an antioxidant [66]. A genus known as Carissa is indigenous to tropical and subtropical regions of Asia, Australia, and Africa. Dhatwalia et al. (2021) examined 155 research papers as part of an updated review. The carissa fruit's abundance in dietary fiber, lipids, proteins, carbs, vitamin C, and other macro- and micronutrients has been verified [67]. Novel tactics are being developed to meet the ongoing demand for new natural antibiotics in order to counteract the rising levels of antibiotic resistance. By utilizing the cutting-edge MS/MS-based molecular networking approach, Wang et al.'s study from 2021 sought to examine amicoumacin antibiotics from the desert-derived *Bacillus subtilis* PJS [68]. Methicillin-sensitive and resistant Staphylococcus epidermidis showed high antibacterial activity against hetiamacin E at 2–4 g/mL, while Staphylococcus aureus showed strong antibacterial activity against hetiamacin E at 8–16 g/mL. At 32 g/mL, hetiamacin F showed some antibacterial activity against Staphylococcus species. According to a double-fluorescent protein reporter system [68], both substances were inhibitors of protein production [68]. The division of naturally occurring bioactive substances into tiny biomolecules, biologics produced by plants, and a newly developed third category known as phytopharmaceutical medications is noteworthy. The ethno-pharmacological methodology, which is based on the traditional medical system, is the foundation for the development of phytopharmaceutical drugs. The idea of "one disease, one target drug" is losing favor, and the usage of plant fractions, extracts, and molecules is the new paradigm that offers hope for developing effective medications. This led to the development of a novel idea known as polypharmacology, in which natural compounds with origins ranging widely can interact with numerous human physiological targets [69]. Numerous naturally occurring bioactive chemicals have been identified by researchers from plants, microbes, and animals. Numerous of these substances, including resveratrol, curcumin, bilirubin, and epigallocatechin gallate (EGCG), have pharmacological or biological properties that make them potent anti-disease agents [70]. The limited bioavailability of these natural chemicals, which has made them useless in clinical trials, is one of the main obstacles to their use [71]. Curcumin, for instance, has a limited range of applications due to its low solubility in water (0.0004 mg mL-1 at pH 7.3), and it also tends to deteriorate at physiological or higher pH levels [72]. As a result, curcumin's insolubility, instability, and low bioavailability have limited its use in clinical settings. The biological transport of natural chemicals with proven therapeutic potential can be enhanced by the introduction of innovative drug delivery methods like nanotechnology. For instance, adding natural substances to nanoparticles can improve their

bioavailability, better target them to specific tissues or receptors, and enable the regulated release of substances over extended periods of time. Natural substances can be delivered to tumor locations with greater therapeutic effectiveness by being encapsulated in nanoparticles, as has been adequately demonstrated in the literature for curcumin [73], flavonoids [74], and traditional Chinese medicines [75]. Long-term application of these nanocarriers is linked to concerns regarding their degradation, elimination, and toxicity in humans [76] because the majority of reported nanocarriers are synthetic (i.e., polymers, lipids, and inorganic metals) and lack intrinsic therapeutic efficacy. Additionally, the drug accumulation in the tumor is severely reduced by nanocarriers' low drug-loading capabilities (below 10% for the majority of anticancer drugs), which affects their therapeutic efficiency [77]. The new paradigms of naturally derived bioactive compounds are summarized in Table 2.

4 Resource Conservation

India has long been regarded as a treasure house of plant genetic resources or as the botanical garden due to its inimitable agro-climatic conditions and regional geography. India is observed as one of the top 12 countries with the most miscellany in the world. More than 8000 different kinds of plants are included in our herbal paragon, which accounts for over 50% of all higher-blooming plant species in India. The tropical forests of the Western Ghats are home to about 70% of the nation's medicinal plants. However, research now available indicates about 1800 species are employed in classical Indian medical systems (IMS). The growing market for herbal products has enormous potential for the economic growth of the Indian region. The use of herbs in IMS as a source of food, medicine, fragrance, flavor, colors, and other products is on the rise. 95% of the therapeutic plants utilized in the Indian herbal business today are thought to

Table 2 New Paradigms of Naturally Derived Bioactive Compounds

Paradigms	Description	Name of crude drug	Bioactive compounds	Therapeutic applications
Single molecules	Plant biosynthesizes signaling molecules such as growth regulators. They also produce alkaloids, terpenoids, and phenylpropanoids, which are important in a variety of developmental and defense processes. These molecules, which are frequently referred to as tiny molecules, are extremely important in controlling the life cycle of plants. These tiny chemicals are secreted when a plant is under stress to defend it from infections, colds, or UV rays [78]	*Hyperian perfotum*	Hypericin	Immunogenic cell death inducer [79]
		Lithospermum erythrorhizon	Shikonin	Immunogenic cell death inducer [80]
		Scutellaria baicalensis	Wogonin	Immunogenic cell death inducer [81]
Naturally derived bioactive-made biologics	In contrast to conventional methods based on in vitro models, which are reliant on exclusive facilities, these bioactive-made biologics (BMBs) are an effective, safer, and more affordable platform to generate therapeutic proteins. The patient's ability to obtain medications has also been aided by PMBs. These plant-produced proteins can be used to create a variety of life-saving medications [82]	Genetically engineered carrot Cells produce enzyme Taliglucerase Alfa	–	Gaucher's disease [83]
Phytopharmaceutical drugs	PPD extracts were enriched with polyphenolic compounds that exhibited specific pharmacological properties against a wide range of human health issues, including allergy, inflammation, and diabetes. In order to treat and prevent disease, the manufacture of herbal drugs frequently lacks adequate control and regulation [84]	*Artemisia annua*	Artemisinin	Type I diabetes and cancer [85]
		Coleus forskohlii	Forskolin	Antiglaucoma drug [86]
		Genista tinctoria L.	Genistein	Anticancer, Alzheimer's disease [87]
		Trigonella foenum-graceum L., trigonelline	Diosgenin	Antidiabetic, anti-conception [88]
		Vitis vinifera L.	Resveratrol	Chemotherapeutic, antidiabetic, antioxidant [89]
Novel drug delivery	The utilization of bioactive, molecules from a diversity of ecology to combat metabolic disorders and other medical ailments. Bioactive, natural compound-derived nanocarriers are anticipated to display intrinsic therapeutic efficacy and greater biocompatibility than drug delivery systems developed from synthetic materials [90]	Indian spice turmeric (Curcuma longa)	Curcumin nanocarriers	Anti-inflammation Anticancer Anti-angiogenic [91]

have been foraged from the wild. Every year, 1.65 lakh acres of forest are cleared and 500,000 tons of dry material are indiscriminately harvested using damaging methods. There is a significant depletion of plant genetic resource wealth, with many of them going extinct day by day, as a result of the rise in population, rapid expansion of land used for food and commercial crops, deforestation, expansion of urban areas, establishment of industries in rural areas, etc. [92, 93]. In reality, during the last few decades, the demand for wild resources has increased by 8–15% yearly [12, 13]. A species' capacity to reproduce is permanently reduced below a certain threshold [94, 95]. There are numerous unique sets of guidelines for the preservation of medicinal plants, including offering both in situ and ex situ conservation. Innate reserves and desolate are typical examples of preserving the efficacy of plants, whereas national parks and seed gene banks are major paradigms for ex situ conservation and future replanting [96, 97].

4.1 Endangered Medicinal Plants

Due to overuse and habitat destruction, many significant and beneficial plant species are on the verge of extinction. A lot of the medicinal plants have become endangered in their natural habitats, and more than 95% of them are gathered from the wild. It is necessary to promote the growth and cultivation of these plants [98].

4.2 Factors Responsible for Exploitation of Medicinal Plants and Resources Thereof

4.2.1 Environmental Factors

1. Annual rainfall has reduced over the past few years, which has affected the health of many herbaceous plants during the summer.
2. Over the past 20 years, deforestation has been documented. The main causes of the decline in the area covered by important woods include the expansion of agriculture, logging, firewood collecting, heavy wood collection, heavy grazing, etc. Due to deforestation efforts, numerous valuable wild medicinal plant species are eliminated or reduced every year.
3. In forests, siltation of water bodies has reduced their ability to hold water, which has led to the depletion of subsurface water.
4. In woods and other regions, the number of honey bee colonies has decreased by 50–60%. Reduced seed set and seed dissemination have been seen as a result of pollinator loss [99].

4.2.2 Activities for Modernization and Development

1. Due to submersion, it has been observed that many species of medicinal plants are disappearing from forests. For instance, the Maradavally forest serves as the catchment area for the Linganamakki Dam, the primary reservoir used in Karnataka for irrigation and power production. Nearly 10 sq. km. of forest were submerged during the monsoon, which led to the extinction of important medicinal plant species.
2. The Devarayanadurga forest in Karnataka has suffered significant harm as a result of road expansion, power line installation, and building construction.
3. Monoculture plantations completely undermine the stability and complexity of the natural world, which leads to a loss of medicinal plants. For instance, many woodlands contain eucalyptus and acacia species.
4. Forestland encroachments have reached troublesome heights. The agricultural methods utilized on highly sloppy lands have also resulted in soil erosion and a loss in the riches of medicinal plants, in addition to the fall of trees and clearance of vegetation.
5. There is a widespread gathering of medicinal plants from the forests. Unorganized forest collectors were responsible for the collection, and they afterward sold the product to a contractor at a price set by the latter. However, illegal gathering has now been somewhat controlled as a result of the awareness raised by the "Local Traditional Medical Practitioners Association" members.

4.3 Recent Updates on Strategies for Conservation of Medicinal and Aromatic Plants

World Conservation Strategy's main objectives for biodiversity conservation:

1. Preservation of vital ecological systems and processes that are necessary for human survival and economic activity.
2. The protection of genetic variety and species, as well as the sustainable use of ecosystems and species that support millions of rural communities and important businesses.

Scientific methods and social initiatives can both be used to address the conservation of wild medicinal plants or any other threatened species [100]. The three basic scientific conservation techniques are i. legislation, ii. in situ conservation, and iii. ex situ conservation (Fig. 2).

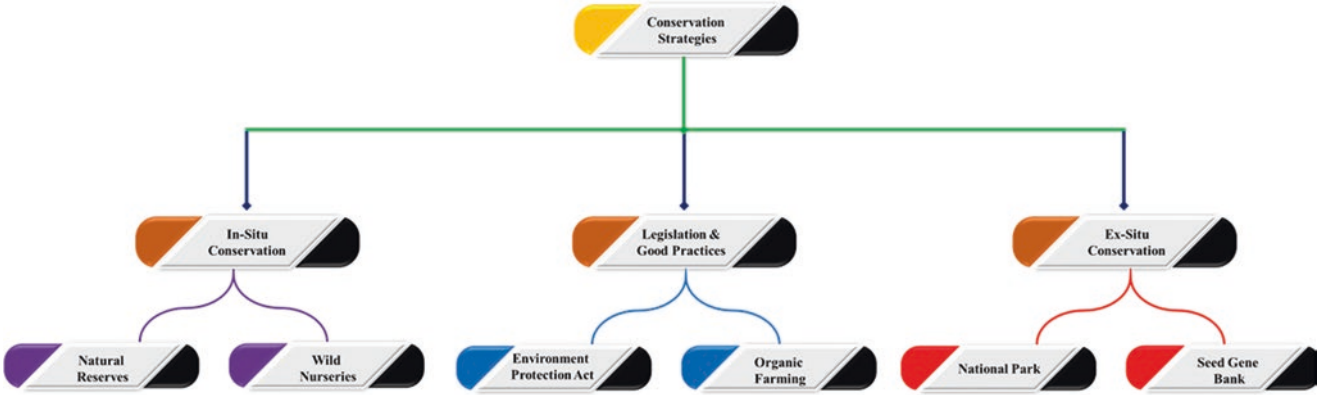

Fig. 2 Resource and conservation strategies

4.3.1 In Situ Conservation

The majority of medicinal plants are endemic species and their therapeutic benefits mostly result from the existence of secondary metabolites, which are compounds that react to stimuli in their natural settings but may not express themselves in cultured environments [97, 101]. We can preserve natural communities and indigenous species while preserving their rich web of relationships thanks to in situ conservation of complete communities [102]. Furthermore, in situ conservation reinforces the connection between resource protection and sustainable use [103] and expands the amount of diversity that can be preserved [104]. Around the world, in situ conservation initiatives have concentrated on creating protected areas and using an ecosystem-oriented strategy rather than a species-oriented one [105]. Rules, regulations, and potential compliance of medicinal plants within growth habitats are necessary for successful in situ conservation [106]. The loss of medicinal plant resources is largely due to habitat deterioration and destruction [107]. To maintain and restore biodiversity, natural reserves were established as protected areas for significant wild resources [108, 109]. More than 12,700 protected areas have been established globally, covering 13.2 million km2, or 8.81% of the land area of the planet [110]. It is necessary to evaluate the contributions and ecosystem functions of each habitat if we want to conserve medicinal plants through the protection of important natural habitats [111]. However, due to financial constraints and competing land uses, it is impractical to designate every natural wild plant habitat as a protected area [112]. In a protected region, a natural habitat, or a location that is only a short distance from where the plants naturally grow, a wild nursery is built for the species-oriented cultivation and domestication of endangered medicinal plants [113]. Although overexploitation, habitat destruction, and invasive species have severely harmed the populations of many wild species, wild nurseries can offer a practical solution for the in situ conservation of medicinal plants that are endemic, threatened, and in demand [114].

4.3.2 Legislation and Good Practices

In India, there are no specific laws or rules protecting the medicinal plants that thrive in forests. There are forestry-related regulations in place that address conservation. The regulations namely (i) Forest Act, 1927, (ii) Wildlife (Protection) Act 1972 and Wildlife (Protection) Amendment Act 1991, (iii) Forest (Conservation) Act, 1980, (iv) Environment Protection Act, 1986, (v) National Forest Policy, 1988, (vi) National Biodiversity Act, 2002, and (vii) The Scheduled Tribes and Other Traditional Forest Dwellers Act, 2006 were created by the Indian government to safeguard forests and, in some cases, the wild herbal flora [98].

In order to control production, guarantee quality, and make it easier to standardize herbal medicines, good agricultural practices (GAP) for medicinal plants have been developed [115]. By using the available knowledge to address numerous issues, a GAP strategy ensures high quality and safety [116]. GAP cover a wide range of topics, including the manufacturing facilities, storage facility, quality characteristics of weed and undesired material detection, morpho-anatomical authentication, identification of bioactive chemicals, and inspection of metal elements [117]. The adoption of GAP is actively supported by many nations. In places where these medicinal plants are customarily grown, for instance, Chinese authorities have supported GAP for the growth of regularly used herbal medications [118]. Similarly, organic farming methods have also drawn more attention due to their capacity to develop integrated, compassionate, and economically and environmentally sustainable agricultural systems. The growth of medicinal plants and the production of important compounds are substantially impacted by the application of organic fertilizers, which continuously supply soil nutrients and enhance soil stability. For instance, when

organic fertilizers were used, Chrysanthemum balsamita's biomass yield increased and its essential oil concentration was high in comparison with plants that did not get organic fertilizers [119]. In reality, damaging harvesting frequently causes resource exhaustion and even species extinction for medicinal plants with low abundance and sluggish growth [120]. Therefore, it is important to think about how to use medicinal herbs sustainably and to develop good harvesting procedures. Harvesting the roots and entire plants does greater harm to medicinal plants (such as herbs, shrubs, and trees) than only taking their leaves, flowers, or buds. Using the leaves of the plants as a medicine can be a safe substitute for herbal medications comprised of whole plants or roots. For instance, Wang et al. [121] found that ginseng leaf stem and root extracts have comparable pharmacological activity, but the leaf stem has the advantage of being a more dependable supply of the herb. Furthermore, advances in tissue culture and the fermentation of medicinal plants have created new opportunities for the large-scale and extremely efficient production of desirable bioactive compounds. Genetic engineering has also made it feasible to synthesize natural products on a large scale. A promising alternative for the generation of uncommon and highly valuable secondary metabolites that are significant from a medicinal standpoint is tissue culture, which includes plant cell and transgenic hairy root culture [122]. Higher regeneration rates can be encouraged by micropropagation using tissue-encapsulated propagules, in addition to storage and transportation conveniences [120]. Synthetic seed technology, which is described as artificially encapsulated somatic embryos (or other tissues) that might be utilized for cultivation in vitro or ex vitro, is a workable option when the quantities of natural seeds are insufficient for propagation [123, 124]. The time needed for breeding may then be greatly reduced by adopting molecular marker-based methodologies applied at the genetic level to carry out breeding improvements [120, 122, 123].

4.3.3 Ex Situ Conservation

Ex situ conservation, which is frequently an immediate action done to conserve medicinal plant resources, aims to nurture and naturalize threatened species to ensure their continuous survival and occasionally to create huge quantities of planting material required in the development of medications [125, 126]. In addition to maintaining high potency when grown in gardens far from their natural habitats, many species of formerly wild medicinal plants can also have their reproductive components carefully chosen and saved in seed banks for future replanting [19]. However, the idea of a national park is crucial for ex situ preservation [127], and they can preserve ecosystems to help rare and endangered plant species survive [110]. Despite the fact that living collections typically only contain a small number of specimens of each species and are therefore ineffective for genetic conservation [128], arboreta has a number of distinctive qualities. They often have taxonomically and ecologically diverse flora and involve a large range of plant species that are grown together under similar conditions [129]. via the creation of propagation and culture guidelines, as well as via the implementation of domestication and variety breeding programs, arboreta can play a further role in the conservation of medicinal plants [130]. However, seed banks are advised to help maintain the ecological species since they provide a superior means of storing the genetic diversity [131, 132].

5 Conclusion

The recent development, implementation, applications, and interrelationship of intellectual property rights over naturally derived bioactive compounds and resource conservation thereof have been discussed and explored in the present chapter. Appropriate examples have also been used to support the topic's relevance in each segment. In conclusion, it is warranted for all researchers, academicians, and businesspeople to resurrect and promote the study of natural remedies for health care, as well as to compensate the traditional resources by abiding by strict rules, regulations, laws, and policies, and to periodically update the strategies for conserving the nation's herbal wealth. The awareness and comprehension of IPR, naturally occurring substances, and resource conservation will undoubtedly pave the road forward for global health.

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ADVANCING FOOD SCIENCE: EXPLORING AGRICULTURAL PRODUCTION, PROCESSING, AND NUTRITIONAL IMPLICATIONS FOR A NOURISHED AND SUSTAINABLE FUTURE

Abstract

Food science, as an interdisciplinary field, delves into the intricate complexities of food and its profound impact on human health and well-being. This chapter presents a comprehensive exploration of various facets within food science, with a particular emphasis on its critical role in agricultural production, food processing, composition analysis, and nutritional implications. Understanding the intricate processes involved in cultivating and handling crops and livestock is paramount for food scientists to ensure a steady supply of safe and high-quality raw materials for food production. Through a range of sophisticated food processing techniques, these experts preserve the nutritional value and uphold food safety standards, thus contributing to the delivery of wholesome and nourishing products to consumers. The analysis of nutritional composition in different food items uncovers valuable insights into their inherent health benefits and potential risks, consequently enabling evidence-based dietary recommendations to enhance overall well-being. Regulatory guidelines set forth by esteemed bodies like the (FSSAI), the (WHO), and the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) play a pivotal role in establishing international standards for food safety and quality. These guidelines guide the efforts of food scientists in their mission to promote public health and ensure global food safety. Moreover, food science actively embraces pioneering innovations such as nanotechnology, functional foods, and

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sustainable practices, offering promising solutions to tackle the pressing challenges facing the global food industry. Emphasizing sustainable food systems and minimizing food waste, food science contributes significantly to fostering a resilient and responsible food environment. By amalgamating the power of cutting-edge research, collaborative endeavors, and adherence to stringent global standards, food science lays the groundwork for a nourished and sustainable future for both humanity and the planet.

Keywords: Food Science; Health; Agricultural Production; Food Processing; Nutrition.

I. INTRODUCTION

Food is an essential aspect of human life, providing sustenance, satisfaction/ gratification, and cultural significance. However, beyond its basic role as a source of energy, food holds a fascinating and complex scientific nature. The study of food science explore into the understanding of the physical, chemical, and biological properties of food and its transformation from raw materials to the final edible product. It is an interdisciplinary field that draws knowledge from various branches, including chemistry, biology, physics, engineering, and nutrition. Food science is an interdisciplinary field that investigates into the complex food, exploring its physical, chemical, and biological properties from farm to fork. This scientific discipline plays a pivotal role in understanding the agricultural production, processing, and distribution of food, while also examining the nutritional aspects that impact human health and well-being. By combining insights from various scientific domains, food science strives to enhance the safety, quality, and nutritional value of the food we consume daily.

The study of food science is motivated by several critical factors that impact individuals and society as a whole. Firstly, ensuring food safety is paramount to safeguard public health from food borne illnesses and contamination. Secondly, understanding the factors that influence food quality allows us to meet consumer expectations while preserving nutritional attributes. Additionally, as the global population grows, food science addresses the challenge of food preservation to prevent waste and enhance food security. Furthermore, researchers and food technologists continuously work to develop innovative food products that cater to evolving consumer preferences while promoting health and sustainability through informed nutrition **[1-4]**.

Agricultural production and food processing involve complex chemical and biological processes that can impact the composition and safety of food products. Quality control in agriculture encompasses various aspects, such as soil health, crop yield, pest and disease management, water management, and sustainable farming practices. Assessing these parameters is crucial to ensure optimal conditions for plant growth, minimize crop losses, and enhance agricultural productivity. Similarly, in the realm of nutrition, ensuring the nutritional quality of food products is essential to meet the dietary requirements of individuals and maintain overall health and well-being **[5-10]**.

Quality control in nutrition involves evaluating the presence and levels of essential nutrients, assessing bioavailability, promoting nutritional balance, and encouraging dietary diversity. These measures aid in providing consumers with safe and nutritious food choices. To achieve accurate and reliable results in quality control, advanced analytical techniques are employed. AAS, ICP-MS, and ICP-OES are powerful tools used in the analysis of trace elements and metals in agricultural products and food items. AAS allows the quantitative determination of specific elements through absorption spectroscopy, while ICP-MS and ICP-OES offer sensitive multi-element analysis capabilities, making them invaluable in assessing nutrient content, detecting contaminants, and ensuring food safety. Throughout this chapter, we will explore the principles and applications of AAS, ICP-MS, and ICP-OES in agriculture and nutrition quality control. Understanding these analytical techniques and their role in monitoring key parameters empowers stakeholders in the food industry, regulatory bodies, and research communities to make informed decisions, uphold quality standards, and

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contribute to a healthier and more sustainable food system. By harnessing the potential of AAS, ICP-MS, and ICP-OES, we pave the way for enhancing agricultural productivity and ensuring nutritious food for a thriving global population **[11-18]**.

The FSSAI is an autonomous regulatory body established by the Government of India to oversee and regulate food safety and hygiene. FSSAI's guidelines encompass various aspects, including food safety standards, labeling, packaging, and quality control. These guidelines aim to ensure that all food products in the Indian market meet stringent safety and quality standards, providing consumers with safe and authentic food choices. FSSAI also plays a vital role in promoting food science research and innovation to address emerging food safety challenges **[19]**.

As a specialized agency of the United Nations, WHO sets international standards and guidelines for nutrition and food safety, with a focus on promoting healthy diets, addressing malnutrition, and preventing foodborne diseases that significantly impact human health. Their recommendations help countries develop robust food safety systems, implement effective food control measures, and create policies to improve public health through nutrition. WHO also emphasizes the importance of collaboration between nations to share knowledge and best practices in food science and safety, fostering a global effort to ensure the well-being of all individuals and communities throughout the globe **[20]**.

While primarily known for its role in pharmaceuticals, ICH also plays a major part in the field of food science. ICH's guidelines address the quality, safety, and efficacy of food additives and ingredients used in the development of food products. These guidelines help maintain consistent global standards for food safety and facilitate international trade by ensuring the harmonization of regulations related to food science.

Moreover, food science is intricately linked to human nutrition and health. Understanding the nutritional content of foods and their effects on the human body is crucial in developing evidence-based dietary guidelines, combating malnutrition, and promoting overall well-being **[21]**.

Another key motivation for studying food science lies in addressing global challenges related to food production and distribution. With a burgeoning/promising/growing global population, food security becomes a pressing concern. Food science plays a fundamental role in developing efficient and sustainable food production methods, reducing food waste, and ensuring equitable access to nourishment for all**[22,23]**.

The field of agriculture and nutrition plays a fundamental role in ensuring the sustenance and well-being of the global population. As the demand for safe and nutritious food continues to rise, it becomes imperative to implement effective quality control measures to monitor and safeguard the quality and nutritional value of agricultural produce and food products. This chapter delves into the significance of quality control parameters in agriculture and nutrition, with a particular focus on the analytical techniques of Atomic Absorption Spectrometry (AAS), Inductively Coupled Plasma Mass Spectrometry (ICP-MS), and Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES).

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1. Agricultural and Nutritional Aspects Related to Food Science: [24-28]

- **Agricultural Production:** The foundation of food science lies in understanding the cultivation, harvesting, and post-harvest handling of crops and livestock. Techniques such as crop breeding, irrigation, and sustainable farming practices directly impact the availability and quality of raw materials for food production.

- **Food Processing:** Once harvested, raw agricultural products undergo various processing methods, including cleaning, milling, cooking, and packaging. Food science ensures that these processes maintain the nutritional value and safety of the final product.

- **Food Composition:** Analyzing the nutritional composition of different foods is vital in determining their health benefits and potential risks. Food scientists assess the presence of macronutrients, micronutrients, vitamins, minerals, and bioactive compounds.

- **Food and Health:** The study of nutrition and dietetics falls under the umbrella of food science. Researchers investigate how specific nutrients affect the human body, helping prevent diseases and promote overall well-being.

- **Food Safety and Quality Assurance:** Food science plays a critical role in ensuring the safety and quality of food products. Food scientists work to identify potential contaminants and hazards in the food supply chain, implementing measures to prevent food borne illnesses. They also develop and optimize food processing techniques to maintain product quality while extending shelf life, reducing food waste, and meeting consumer demands.

- **Food Preservation and Storage:** Food scientists investigate various preservation methods such as canning, freezing, drying, and fermentation to extend the shelf life of food products. These techniques not only prevent spoilage but also retain the nutritional content of foods, allowing consumers to access a wide range of produce throughout the year.

- **Sensory Evaluation:** Understanding the sensory aspects of food is crucial in developing products that appeal to consumers' tastes and preferences. Food scientists employ sensory evaluation techniques to assess attributes like taste, texture, aroma, and appearance, enabling the creation of products that satisfy both sensory pleasure and nutritional needs.

- **Food Additives and Ingredients:** Food science explores the use of additives and ingredients to improve food products' quality, appearance, and stability. Food scientists evaluate the safety and efficacy of additives, ensuring they comply with regulations and contribute positively to the overall consumer experience.

- **Food Packaging Technology:** Packaging plays a crucial role in preserving food quality, protecting it from physical damage, and preventing contamination. Food

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scientists study packaging materials and technologies, ensuring they meet safety standards and maintain the freshness of the product throughout its shelf life.

- **Food Nanotechnology:** An emerging area of food science, nanotechnology explores the application of nano particles in food production and processing. It offers opportunities to improve food quality, enhance nutrient delivery, and develop innovative functional foods with potential health benefits.

- **Sustainable Food Systems:** Food science is at the forefront of promoting sustainable practices in agriculture and food production. Researchers investigate eco-friendly techniques, reduced food waste strategies, and sustainable sourcing to address environmental challenges and create a more resilient and sustainable food system.

- **Novel Food Processing Technologies:** Food science embraces cutting-edge technologies such as high-pressure processing, pulsed electric field, and non-thermal processing methods. These innovative techniques preserve the nutritional integrity of foods while ensuring safety and extending shelf life.

- **Functional Foods:** Food scientists explore the concept of functional foods, which offer additional health benefits beyond basic nutrition. These foods are formulated with bioactive compounds, pro-biotics, or nutraceuticals that may promote specific health outcomes, such as improved digestion or immune support.

- **Nutrigenomics:** The integration of genetics and nutrition is a burgeoning field in food science. Nutrigenomics studies how individual genetic variations influence responses to specific nutrients, leading to personalized dietary recommendations for optimal health and disease prevention.

2. Quality Parameters of Agriculture: [29-38]

- **Soil Health:** Assessing soil fertility, pH levels, and nutrient content to ensure optimal conditions for plant growth.
- **Crop Yield:** Measuring the quantity and quality of crop production to maximize output and efficiency.
- **Pest and Disease Management:** Implementing strategies to control pests and diseases to minimize crop losses and maintain product quality.
- **Water Management:** Efficiently managing water resources to ensure adequate irrigation while minimizing wastage.
- **Sustainable Farming** Practices: Promoting environmentally friendly and sustainable agricultural methods to protect ecosystems and natural resources.
- **Genetic Diversity:** Preserving genetic diversity in crops to enhance resilience and adaptability to changing environmental conditions.
- **Crop Rotation:** Practicing crop rotation to improve soil health, control pests, and reduce the risk of soil degradation.
- **Organic Farming:** Emphasizing organic farming practices to avoid the use of synthetic chemicals and promote healthier, chemical-free produce.

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- **Use of GMOs:** Monitoring and regulating the use of genetically modified organisms to ensure safety and adherence to guidelines.
- **Certification and Standards:** Adhering to agricultural certification and quality standards to guarantee the authenticity and traceability of produce.

3. Quality Parameters of Nutrition:[39-48]

- **Nutrient Content:** Evaluating the presence and levels of essential nutrients in food to meet dietary requirements.
- **Bioavailability:** Assessing the extent to which nutrients can be absorbed and utilized by the human body.
- **Nutritional Balance:** Ensuring a balanced diet that provides adequate amounts of macronutrients (proteins, carbohydrates, and fats) and micronutrients (vitamins and minerals).
- **Nutritional Density:** Promoting foods that offer a high concentration of nutrients relative to their caloric content.
- **Dietary Diversity:** Encouraging a diverse diet to ensure the intake of a wide range of nutrients and prevent deficiencies.
- **Nutritional Guidelines:** Following dietary guidelines provided by health authorities to promote optimal nutrition and well-being.
- **Nutritional Labeling:** Providing clear and accurate nutritional information on food packaging to help consumers make informed choices.
- **Special Dietary Needs:** Addressing specific nutritional requirements for different age groups, medical conditions, and lifestyle preferences.
- **Nutritional Education:** Educating the public about the importance of balanced nutrition and healthy eating habits.
- **Impact of Food Processing:** Understanding the effect of various food processing techniques on nutrient retention and bioavailability.

4. Quality Parameters of Food Science:[49-68]

- **Safety:** Ensuring that food products are free from harmful contaminants and pathogens to safeguard public health.
- **Nutritional Content:** Assessing the presence of essential nutrients, vitamins, minerals, and bioactive compounds to promote optimal nutrition and well-being.
- **Sensory Attributes:** Evaluating taste, texture, aroma, appearance, and overall sensory appeal to enhance consumer satisfaction and acceptability.
- **Shelf Life:** Determining the stability and longevity of food products, preventing spoilage and maintaining quality over time.
- **Microbiological Purity:** Ensuring that food is free from harmful microorganisms that could cause foodborne illnesses.
- **Processing Techniques:** Implementing food processing methods that preserve nutritional value, taste, and safety while meeting consumer demands.
- **Authenticity and Traceability:** Verifying the origin and authenticity of food products through traceability systems to prevent food fraud.

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- **Labeling and Packaging:** Ensuring accurate and informative labeling to provide consumers with essential information about the product and its contents.
- **Sustainability:** Considering the environmental impact of food production and distribution to promote eco-friendly practices and reduce waste.
- **Allergen Management:** Managing and clearly labeling potential allergens in food products to protect consumers with allergies.
- **Regulatory Compliance:** Adhering to national and international food safety and quality regulations to maintain high standards.
- **Process Efficiency:** Implementing efficient and cost-effective food production processes to optimize resources and reduce waste.
- **Traceability and Recall Systems:** Developing systems to trace the origin of raw materials and finished products and enabling swift product recalls in case of safety issues.
- **Packaging Integrity:** Ensuring that food packaging protects the product from contamination and maintains its quality throughout the supply chain.
- **Continuous Improvement:** Engaging in research and innovation to enhance food safety, quality, and nutritional value continuously.
- **Sustainability Practices:** Incorporating sustainable and environmentally friendly practices in the food production and distribution chain to reduce the ecological footprint.
- **Consumer Feedback and Engagement:** Actively seeking and considering consumer feedback to improve products and processes based on consumer preferences and needs.
- **Food Waste Reduction:** Implementing strategies to minimize food waste at various stages of the supply chain to promote resource conservation.
- **Product Innovation:** Driving innovation to create new and healthier food products that cater to evolving consumer preferences and health concerns.
- **Food Safety Training and Education:** Providing food safety training and education to food handlers, processors, and consumers to enhance overall awareness and compliance with food safety standards.

II. CONCLUSION

Food science stands at the forefront of ensuring the world's sustenance through a deep understanding of the complexities of food. This interdisciplinary field plays a vital role in various aspects of the food supply chain, from agricultural production to the nutritional content of the final product. The guidelines set forth by regulatory bodies such as FSSAI, WHO, and ICH emphasizes the importance of safety, quality, and nutrition in the global food landscape. Food science continually strives to address challenges in food safety, optimize food processing techniques, and promote healthier dietary choices. Agricultural and nutritional aspects are at the core of food science, encompassing agricultural production, food processing, food composition, and the relationship between food and health. Understanding soil health, crop yield, pest management, and sustainable farming practices ensures an efficient and environmentally friendly agricultural system. Analyzing the nutritional content of foods helps develop evidence-based dietary guidelines, promoting overall well-being. Food science also addresses global challenges such as food preservation, food waste

reduction, and the development of innovative food products to cater to diverse consumer needs.

By exploring the composition and nutritional value of different foods, food scientists contribute to enhancing human health and well-being. Moreover, the advancements in food science technology and innovative approaches, such as nanotechnology and functional foods, offer promising avenues to meet the demands of a growing population while addressing environmental concerns. With a strong focus on sustainability, food science drives research to develop eco-friendly practices and reduce food waste, creating a more resilient and responsible food system. Through continuous research, collaboration, and adherence to international standards, food science holds the key to fostering a safer, healthier, and more nourishing future for individuals and communities worldwide.

As we investigate further into the realms of food science, it is imperative to recognize the critical role it plays in shaping our diets, ensuring food safety, and creating a sustainable and thriving global food landscape. Together, the efforts of food scientists, regulatory bodies, and stakeholders pave the way for a more informed, sustainable, and nourished world, where food not only delights our taste buds but also nurtures our bodies and sustains the planet for generations to come.

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ULCERATIVE COLITIS: A BRIEF REVIEW ON NUTRITION MANAGEMENT

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ABSTRACT

Ulcerative colitis is a chronic inflammatory bowel disease of unknown aetiology affecting the colon and rectum having high morbidity and recurring symptoms. Multiple factors which contribute to Ulcerative colitis include genetic background, environmental factors, luminal factors and mucosal immune dysregulation. Up to 25% of ulcerative colitis patients experience extraintestinal symptoms. The diagnosis is made preferably by endoscopic techniques with the support of laboratory and histopathological testing. The severity and extent of the condition determines the course of treatment. The present study shows impairments in cognitive functioning in inflammatory bowel disease-ulcerative colitis patients. Proctitis is treated with rectal treatment using 5-aminosalicylic acid molecules. Corticosteroids and oral 5-aminosalicylic acid derivatives are needed to treat more severe illness. Steroids' suitability for long-term treatment is constrained by their side effects. For those with ulcerative colitis, the future is anticipated to provide a variety of novel therapeutic alternatives like nutritional management along with diet therapy with the possibility of a more individualised treatment strategy.

INTRODUCTION

The human intestine, a muscular tube of the digestive system that extends from the stomach to the anus, is the hub of the human body's ability to absorb and acquire nourishment. This incorporates the generation of the hormones and enzymes that are essential for both healthy human physiology and the digestion of food (e.g., cholecystokinin that stimulates the secretion of pancreatic enzymes and bile). Numerous conditions impair the colon's ability to function physiologically normally [1]. The term "inflammatory bowel disease" is used to refer to conditions that cause chronic inflammation of the digestive tract. These conditions include ulcerative colitis (ulcerative colitis) and Crohn's disease (CD) [2,3]. Ulcerative colitis is an idiopathic, chronic inflammatory illness of the colon that is typically marked by recurrent and remitting mucosal inflammation, originating in the rectum and extending to proximal portions of the colon. Inflammation of the intestine may start appearing from the stage of proctitis extending to left-sided colitis to extensive colitis; however there is no clear evidence of their interdependence. The hallmark sign of the illness is bloody defecation [3,4]. Although the ulcers are observed to be limited in the colon, there are some systemic complications associated with the pathophysiology of ulcerative colitis. An inappropriate inflammatory response to intestinal microorganisms in a genetically vulnerable host leads to a condition known as inflammatory bowel disease. Despite the fact that the exact origin of inflammatory bowel disease is unknown, genetically predisposed people appear to have an abnormal mucosal immune response to symbiotic gut flora, which causes intestinal inflammation [5]. From history till date the aetiology of ulcerative colitis is not really postulated in the proper manner and has an unknown aetiology. Samuel Wilks first described the ulcerative colitis in 1859[6]. Wilks and Moxon reported a case of bloody colitis more than a century ago (1875) which was not caused by dysenteric pathogens. Throughout the years 1938 to 1954, sulphasalazine (SASP) was the sole medication available for the treatment of ulcerative colitis. SASP, made up of a salicylate-5-aminosalicylic acid (5-ASA) and a sulphonamide sulphapyridine, was employed by Nanna Svartz. It was hypothesised that because of its antibacterial activity, bacteria may have played a role in the development of ulcerative colitis. The most recent meta-analysis demonstrated that the parent compound is more effective than 5-ASA derivatives at preventing ulcerative colitis relapses [7,8]. Ulcerative colitis is classified by extent of colonic involvement; Proctitis, Left-sided colitis and Extensive colitis. Proctitis is an inflammation that spreads in the anal region. Tenesmus and urgency (sensation of incomplete evacuation) are the symptoms of this type of ulcerative colitis in 30–60% of patients. Patients with left-sided colitis or pancolitis (16–45%) typically exhibit proctitis plus diarrhoea, abdominal discomfort, and 10% have paradoxical constipation. Extensive colitis (15-35% of patients) is a disorder characterised by total inflammation of the large intestine and symptoms such as constipation, tiredness, and fever [6]. There are many factors such as geography, age, sex, genetic factors, environmental factors etc which triggers the onset of ulcerative colitis.

The exact cause of ulcerative colitis is not known. However, it is believed that the disease occurs as a result of a complex interplay between genetic and environmental factors. The genetic basis of ulcerative colitis is supported by the fact that the disease is more common in people with a family history of inflammatory bowel disease. Several genes have been identified that increase the risk of developing ulcerative colitis, including genes involved in the immune system, barrier function, and the response to inflammation. Ulcerative colitis progresses by T-helper (Th) 2 cytokines-induced chronic inflammation which causes extensive epithelial apoptosis and ulceration. A balance between immune-suppressive Treg cells and proinflammatory Th17 cells appears to be essential for the development of ulcerative colitis, according to recent studies. Environmental factors that may contribute to the development of ulcerative colitis include a Western diet high in fat and low in fibre, smoking, and the use of non-steroidal anti-inflammatory drugs (NSAIDs). In addition, infections and alterations in the gut microbiome have also been implicated in the development of ulcerative colitis [9,10].

Despite of the advances in understanding of the disease genetically, immunologically and environmental factors, no specific markers are specified that can diagnose ulcerative colitis First order to diagnose ulcerative colitis is to determine the symptoms (diarrhoea and abdominal cramps) which can overlap with inflammatory bowel syndrome and Crohn's disease. Diagnosis is based on a combination of clinical presentation, endoscopic findings, histology, laboratory studies etc., it is also important to define the extent and severity of the inflammation. Endoscopy is considered the gold standard for diagnosing ulcerative colitis, it allows the direct visualization of colon and rectum and can help to determine the extent and severity of inflammation. Biopsy samples may also be taken during this procedure to help confirm the diagnosis. Unlike Crohn's disease, where the surrounding mucosa may not always appear inflamed, ulcers in ulcerative colitis are invariably associated with mucosal inflammation. Laboratory tests include complete blood count (CBC), liver function tests and inflammatory markers such as C-reactive protein (CPR), erythrocyte sedimentation rate (ESR) which

may be elevated. Faecal calprotectin or faecal lactoferrin tests may be more sensitive and would prove to be the specific markers. However only these tests do not support the diagnosis as they can be elevated in intestinal inflammation or infection of any cause. Histopathology is another definitive tool in which the mucosal layer of the colon is studied, some of the features which are observed are epithelial damage with presence of neutrophils, increased transmucosal lamina propria cellularity with basal plasmacytosis, presence of basal lymphoid aggregates and presence of high number of eosinophils [6,11-14].

Ulcerative colitis is more prevalent than Crohn's disease (CD) in all industrialised nations, with a higher frequency in North America and Western Europe in particular. In quite a while, Asia is seeing a rise in ulcerative colitis cases. South Asian immigrants of the second generation in the UK have a greater incidence of ulcerative colitis than people of European descent. The prevalence and incidence of ulcerative colitis vary from 7.6 to 245 cases per 100,000 people per year and 1.2 to 20.3 and 7.2 to 20.3 respectively. Ethnic and ethnic distinctions are more due to environmental effects, eating preferences, and lifestyle than genetic variations. There are no evident gender differences in ulcerative colitis, and its frequency peaked in the second or third decade of life, followed by another peak between the ages of 50 and 80[6, 11, 14].

Causes and Risk Factors

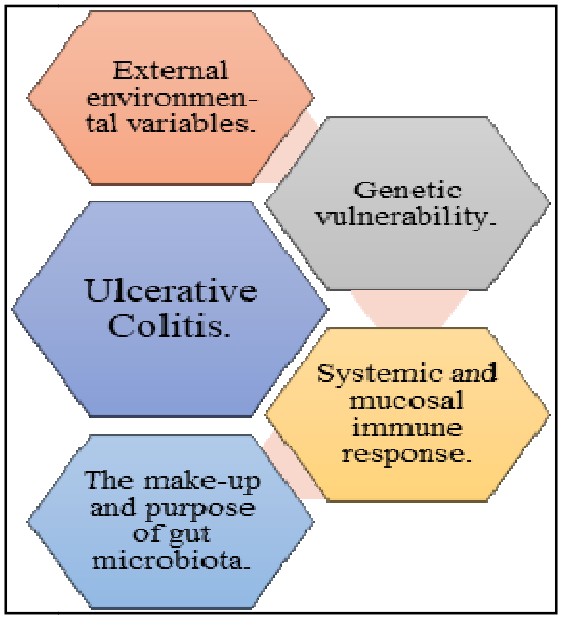

Fig. 32.1 Causes and risks of ulcerative colitis

- Non-steroidal anti-inflammatory drugs (NSAIDs) are believed to cause colitis as well as exacerbate existing disease by increasing permeability and contributing to colonic bleeding.
- The following dietary sources increase the risk: Refined sugar, Starch, Total protein, Mono- and polyunsaturated fats, Sucrose, Fat (especially animal fat), Fast food. [15]
- The presence of *Shigella* or *Shigella* - like toxin, *Salmonella* and *Yersinia* has been investigated as a possible cause of ulcerative colitis. [16]

Complications

Blood loss from the inflamed intestines can cause anaemia in people with more severe disease, which may need iron supplements or even blood transfusions to cure. When the inflammation gets really bad, the colon can very rarely suddenly expand to a huge size. Toxic megacolon is the name of this condition. The symptoms of toxic megacolon are severe illness, including starvation, dehydration, and fever. Surgery is frequently required to prevent colon rupture unless the patient quickly gets better with treatment.

A known side effect of chronic ulcerative colitis is colon cancer. After 8 to 10 years of colitis, the risk for cancer starts to increase considerably. 10% of patients (arthritis) some patients suffer low back pain owing to arthritis of the sacroiliac joints. Occasionally, patients may have painful, red skin nodules (erythema nodosum). Some may have eyes that are sore and inflamed (uveitis, episcleritis). Eye pain or redness are symptoms that demand a doctor's assessment because these specific issues pose a lifelong risk of vision impairment. Ulcerative colitis may be accompanied by conditions affecting the liver and bile ducts. [17]

For a long time, it was thought that muscle hypertrophy, thickening of the muscularis mucosae and muscularis propria, rather than fibrosis, was the cause of ulcerative colitis -associated structures.

Pseudopolyposis of post inflammatory polyps have been connected with ulcerative colitis. These lesions are thought to be caused by ulceration, which damages the muscularis mucosae and allows for the big mucosal tags to be dragged out by the gut's propulsive activity.

It has been long documented that there is a colonic motility abnormality in people with ulcerative colitis, which may play a significant role in the origin of symptoms.

Impaired anorectal function, which causes urgency, tenesmus, and outright incontinence, is a particularly painful form of dysmotility in ulcerative colitis. [18]

PATHOPHYSIOLOGY OF THE DISEASE

The pathophysiology of ulcerative colitis involves a complicated interaction between the intestinal epithelium, the immune system and the gut microbiota. The intestinal epithelium acts as a physical and immunological barrier between the luminal contents and the underlying tissues. The intestinal epithelium is disturbed in ulcerative colitis, allowing luminal bacteria and toxins to infiltrate the intestinal mucosa and cause an inflammatory response [19].

The immune system plays a central role in the pathogenesis of ulcerative colitis. A dysregulated immune response to luminal antigens manifests in an exaggerated inflammatory response in the colon. Pro-inflammatory cytokines such tumour necrosis factor-alpha (TNF-alpha), interleukin-1 (IL-1), and IL-6 are overproduced. These cytokines draw in and prime immune cell populations, such as T cells, B cells, and macrophages, amplifying the inflammatory response.

The intense research studies in this area progresses with satisfactory rate which confirms the clear importance of genetic variables in the pathophysiology of ulcerative colitis. Around 200 genetic loci related with ulcerative colitis have been found through genome-wide association studies (GWAS). Several of these loci are implicated in immunological modulation and intestinal epithelial barrier function. NOD2/CARD15 gene mutations, which encode a pattern recognition receptor involved in bacterial sensing, have been substantially linked to ulcerative colitis [20].

The gut microbiome is also significant in the pathophysiology of ulcerative colitis. In ulcerative colitis, the gut microbiota is dysbiotic, with a decrease in beneficial commensal bacteria and an increase in potentially pathogenic bacteria. This dysbiosis alters the production of anti-inflammatory microbial metabolites such as short-chain fatty acids (SCFAs) and bile acids. This altered microbial metabolite production leads to the development and persistence of inflammation in ulcerative colitis.

In conclusion, ulcerative colitis is a complex disease with multiple factors contributing to its pathogenesis. The disruption of the intestinal epithelium, dysregulated immune response, dysbiosis of the gut microbiota, and genetic factors all play a critical role in the development and persistence of inflammation in ulcerative colitis. A better understanding of the pathophysiology of ulcerative colitis will lead to the development of more effective therapies for this debilitating disease [21-23].

Allopathic Remedies [24]

Classification of drugs used in Ulcerative colitis:

- Amino-salicylates: Sulfasalazine, Mesalamine, Olsalazine, Balsalazide
- Corticosteroids: Prednisolone, Hydrocortisone, Budesonide
- Immunomodulator agents: Azathioprine, 6-mercaptopurine
- Immunosuppressive agents: Methotrexate, Cyclosporine
- Antibiotic: Metronidazole
- Biological agents: Infliximab, Adalimumab, Certolizumab, Natalizumab

Table 32.1 Drugs, their doses and route of administration

Drug	Dose	Route of Administration
Amino-salicylates	2-4 mg/day	Oral, topical (Mesalamine suppositories)
Corticosteroids Hydrocortisone Prednisolone	 Parenteral:100mg/for every 8hr Parenteral: 30mg/ for every 8hr Oral: 30-60 mg	Oral / parenteral
Immunomodulator agents Azathioprine 6-mercaptopurine	 2.5 mg/kg/day 1-1.5 mg/kg/day	Oral / parenteral
Immunosuppressive agents cyclosporine	 4 mg/kg/day	 Oral
Infliximab	5 0r 10 mg/kg	Oral

Future prospective therapies

1. Tacrolimus: A placebo-controlled trial showed that tacrolimus induces remission in refractory ulcerative colitis patients
2. Trichuris suis: Ova or placebo for 12 weeks.
3. Rosiglitazone: A thiazolidinedione used to treat diabetes mellitus, was more effective than placebo in achieving remission in mild to moderately active ulcerative colitis in a placebo-controlled trial.
4. Visilizumab: It is a humanized anti-CD3 monoclonal antibody. It has been studied in a phase I study in severe steroid-refractory ulcerative colitis

NUTRITIONAL MANAGEMENT

Many nutritional deficits are linked to ulcerative colitis. In a clinical investigation involving 46 ulcerative colitis patients, they found that newly diagnosed patients had considerably lower levels of beta-carotene, iron, magnesium, selenium, copper and zinc than controls. Low levels of antioxidants including carotenoids, selenium, and zinc may be related to increased oxidative stress, which causes the inflamed intestinal tissue to consume more antioxidants [25].

The patient with mild to moderate disease who is being handled as an outpatient should not forget about maintaining a well-balanced, healthy diet. Avoiding unneeded dietary limitations is crucial. To prevent diarrhoea, many patients place themselves on extremely restrictive diets. Although Ulcerative Colitis patients have lactose intolerance at the same rate as the general population, routine lactose restrictions are not necessary [26].

Diet

1. Micronutrients

It is challenging to evaluate the status of several micronutrients in ulcerative colitis. There is not much research looking into this, and most of them don't distinguish between ulcerative colitis and Crohn's disease, which affects the small intestine. Malnutrition is widespread in ulcerative colitis, and two-thirds of patients have an iron shortage. Except for iron, there are rarely any overt signs of nutritional deficiencies in ulcerative colitis. Deficiencies are more prone to arise when disease activity is high, when demands and losses are high and intake is low. In ulcerative colitis patients, serum copper concentrations are frequently higher. There have been reports of declining zinc levels in the serum together with declining vitamin A levels [26].

1. **Vitamin A and Carotenoids**

   Retinol and carotenoids are crucial for improving the integrity of the gut mucosa. Ulcerative colitis is characterised by low levels of retinol binding protein (RBP), which has a knock-on effect on serum retinol levels. RBP decreases could be brought on by excessive protein loss from diarrhoea related to Ulcerative colitis.

   Retinoids and carotenoids are crucial in preserving the integrity of the mucosa lining the digestive tract. Vitamin A serum levels are considerably decreased in ulcerative colitis. According to one study, the carotenoid most significantly reduced in ulcerative colitis was beta-carotene. Lycopene and zeaxanthin showed the biggest difference between active disease and remission in ulcerative colitis patients, demonstrating how the degree of disease activity also affected some antioxidant levels to varying degrees. Lycopene dramatically decreased inflammatory symptoms in an animal colitis model.

2. **Vitamin E**

   Some Ulcerative colitis patients have been shown to have low amounts of vitamin E. Low amounts were discovered to only occur during active disease, probably due to the increased oxidative stress seen during inflammation.

3. **Vitamin C**

   An Australian study looked at colonic samples from Ulcerative colitis patients and assessed the levels of decreased and total ascorbic acid in the mucosa. Reduced-ascorbic acid content in mucosa decreased by 41% while total ascorbic acid content dropped by 73%. In the inflamed mucosa of Ulcerative colitis patients, the enzyme reduction of dehydroascorbic acid dropped dramatically, indicating that inflamed mucosa is less able to maintain reduced ascorbic acid concentrations. Inflammatory cells' ability to induce oxidative stress resulted in a considerable reduction of antioxidant buffering capacity, which slowed tissue repair.

4. **Vitamin K**

   Ulcerative colitis has been linked to vitamin K insufficiency, which can cause aberrant prothrombin, according to research. Those who lack vitamin K exhibit significantly reduced amounts of plasma vitamin E.

5. **Vitamin B6 (folic acid)**

   Many factors, such as reduced dietary intake, red cell hemolysis brought on by long-term drug use, persistent diarrhoea, and sulfasalazine therapy, which prevents folate absorption, may have an impact on the folic acid status of ulcerative colitis patients. High homocysteine levels, which are frequently observed in ulcerative colitis patients, may be related to folate insufficiency. It is shown that while folate levels were decreased in ulcerative colitis patients, homocysteine levels were noticeably greater.

6. **Calcium**

   Patients with ulcerative colitis may experience calcium shortage for a number of reasons, such as reduced dietary intake, malabsorption, enteric losses, concomitant vitamin D inadequacy, and corticosteroid therapy.

7. **Iron**

   Due to persistent GI bleeding, iron deficiency and the ensuing anemia are common in ulcerative colitis patients.

   In contrast, it has been found that the mucosal iron concentration is much higher when inflammation is present, at least in part because of an excess formation of free radicals via elevated amounts of free haemoglobin from mucosal ulceration and bleeding. Researchers have found immunoreactivity to the main iron-binding proteins lactoferrin, transferrin, and ferritin in the pre neoplastic colonic mucosa of ulcerative colitis patients. Excessive production of these proteins and consequent iron build up may set off a self-reinforcing loop that exacerbates tissue damage and promotes the development of cancer. In view of greater iron concentrations in intestinal mucosa, iron/supplementation in ulcerative colitis should be avoided if possible

8. **Magnesium**

   Magnesium shortage is common in ulcerative colitis patients, although it is unclear if this is due to the disease, which causes malabsorption and intestinal loss, or a contributing factor from lower intake.

9. **Selenium**

   Similar to magnesium, newly diagnosed ulcerative colitis patients had considerably reduced serum and plasma selenium levels.

10. **Zinc/Metallothionein**

    Zinc levels in ulcerative colitis patients have given inconsistent outcomes. The most recent studies demonstrate a notable decline in serum zinc levels. Colonic inflammation may contribute to decreased local zinc availability in mucosal tissue, which in turn causes a decrease in zinc dependent antioxidant enzymes such metallothionein. Research shows that whereas mucosal zinc and metallothionein concentrations are frequently lowered, particularly at inflammatory locations, there are no changes in plasma zinc levels.

11. **Copper**

    Patients with ulcerative colitis frequently have elevated serum copper levels, which, in times of inflammation, may also be accompanied by elevated levels of ceruloplasmin, the copper's transport molecule. An ongoing cycle of inflammation in ulcerative colitis may occur from an increase in oxidative stress in the colonic mucosa caused by excess copper. Little doses of copper may be recommended as a supplement since zinc supplementation may cause a copper deficit.

2. Specific Nutrients

1. Essential Fatty Acids

Omega-3 and -6 fatty acid compositions in ulcerative colitis patients have been found to vary. These modifications may affect the synthesis of fatty acids by colonic tissue as well as the lipid content of luminal cells' membranes, thereby contributing to the pathogenesis of illness.

2. Short Chain Fatty Acids

SCFA supplementation via butyrate enemas has been the subject of multiple investigations in ulcerative colitis patients because of the crucial role they play in the preservation of colonic integrity and energy metabolism [25].

3. Mediterranean Diet

This is one of the healthier eating plans that is furthermore suggested by ESPEN (European Society for Clinical Nutrition and Metabolism) as an optional method for ulcerative colitis patients. This diet is based on eating a lot of fruits, vegetables, whole grains, legumes, fish, and olive oil, which are all high in monounsaturated and polyunsaturated fatty acids, antioxidants, and dietary fiber. Moderate dairy consumption is also part of the Mediterranean diet, particularly fermented dairy products (such as yoghurt, kefir, and cheese), as well as eggs. Each dish is additionally fortified with 21 herbs and spices, whose antioxidant effects have been scientifically proven, including parsley, thyme, oregano, basil, cumin, cinnamon and turmeric.

Every person, but notably those with inflammatory bowel disease, needs to eat well because, among other things, diet is one of the main elements regulating the gut microbiota. The current Western diet, which is low in dietary fibre and heavy in simple carbohydrates and saturated fats, may affect how the gut microbiota is made up. Due to the critical role that gut microbes play in the body's immune system, dysbiosis can result in a wide range of health issues.

Therefore, the immune system function is compromised as a result of the development of unfavourable microorganisms, which may be stimulated by an inadequate diet. This may then result in an increase in the production of inflammatory factors, which may then stimulate the development of intestinal inflammation. Dietary adjustments can be used to treat certain illnesses. According to studies, eating a Mediterranean-style diet improves the balance between harmful bacteria like *Bifidobacterium* and *Bacteroides fragilis* and helpful ones like *Firmicutes* and *Escherichia coli*. This nutritional strategy may be useful in the therapeutic management of ulcerative colitis patients due to the favourable effects of the Mediterranean diet's products on the gut microbiota's composition [27].

4. Probiotics

When compared to ulcerative colitis patients in remission, ulcerative colitis patients with active disease usually exhibit lower concentrations of facultative organisms and micro-aerobes, as well as obligate anaerobes such *Bifidobacterium*, *Eubacteria*, and *Clostridia*. As a result, scientists have investigated how probiotic supplementation affects ulcerative colitis patients. Some *E. coli* subspecies, *Streptococci*, *Bifidobacterium* and *Lactobacilli* are examples of probiotic bacteria. Since lactic acid production lowers luminal content pH and prevents the formation of putrefactive or dangerous bacteria, probiotics may be useful in ulcerative colitis. Another possibility is the synthesis of bacteriocin, which has a direct antimicrobial effect. A probiotic must be safe and well-tolerated, enter the intestinal tract in a viable state, and cling firmly to the intestinal mucosa in order to be effective [25].

5. Elimination/Hypoallergenic Diet

Food allergies have long been considered a potential cause of ulcerative colitis, with the majority of study concentrating on an allergy to the protein found in cow's milk. Elimination diets appear to have the greatest potential for treating ulcerative colitis, which may be aggravated by other highly allergic foods. Nine people on the elimination diet experienced considerably fewer ulcerative colitis symptoms (mainly diarrhoea and rectal bleeding) compared to nine patients eating normally, according to a small study of 18 ulcerative colitis patients. The elimination diet excluded generally known allergenic items. Citrus fruits, dairy, pork, tomatoes, pineapple, seafood, spiced or curried foods, apples, grapes, and melon were among the foods that appeared to cause symptoms. Four patients in this trial achieved remission on the elimination diet in addition to a reduction in symptoms, and eight months later, despite returning to a regular diet, three of them remained symptom-free. Diet with fibre those with ulcerative colitis may benefit therapeutically. A high-fibre diet may offer protection from disease or relapse, as diets with low fibre content have been linked to an increased risk of ulcerative colitis. This shows that ulcerative colitis patients may benefit more from a high-fibre diet made up of complex carbs rather than refined ones. By increased butyrate synthesis, high-fibre diets may help support beneficial gut flora composition [25].

CONCLUSION

The pathophysiology of Ulcerative colitis, which is characterized by persistent intestinal inflammation, has been hypothesized to be heavily influenced by environmental variables, including nutrition. In an investigation, the only elimination diet associated with a lower clinical relapse rate in ulcerative colitis patients who were in remission at baseline was carrageenan-free. Despite the fact that the elimination of certain foods is based on each participant's self-reported food intolerance, there were some general suggestions about specific food groups/items such dairy products, refined sugar, and beverages. Nonetheless, it is usually noted that foods that are spicy or curried as well as fruits (particularly grapes, melon, and citrus fruits) may result in symptoms. In ulcerative colitis, malnutrition is common; it has a complicated aetiology and is associated with worse results. Enteral nutrition is favoured by modality since it is less expensive, has fewer issues and is just as effective as parenteral nutrition. Many studies have validated the use of enteral formulations to manage disease activity in adult patients. In comparison to drugs, nutritional therapy benefits the people with ulcerative colitis by lowering inflammation, encouraging mucosal repair and enhancing general nutritional status.

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31 CURRICULUM VITAE OF QUERCETIN: A VERSATILE PLANT DERIVED DRUG WITH MULTIPLE THERAPEUTIC EFFECTS

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ABSTRACT

Herbal medicine and their preparations have been widely used for thousands of years in developing and developed countries in the primary health care of society and community. They have great demand due its safety, efficacy with minimum side or adverse effects. Traditional herbal medicines are generally naturally occurring and derived from plants with very less or no requirement of industrial methodology in its final production and used to treat illness, diseases or disorders in local or regional healing practices. Flavonoids are a type of polyphenolic chemical that is found throughout the plant kingdom. Quercetin is a flavonoid that has been employed as a contemporary medication derived from plants and used for a variety of therapeutic purposes. The primary goals of the proposed review effort are to collect and publish detailed information about Quercetin. Many review and research articles from various journal databases were consulted in order to gather information on Quercetin, and data was processed and computed. The proposed review article covers in detail information of Quercetin under different headings like, discovery, pharmacokinetic properties, and physicochemical properties, mechanism of action, synthesis, medicinal values, adverse effect, contraindications, interactions, patents, marketed and novel formulations, patents, future implications, computational data about drug likeness, pharmacokinetic prediction, molecular docking and analytical studies on Quercetin. The proposed review work concludes that Quercetin can be considered as a well-known plant derived drug with multiple therapeutic actions and hence in future this compound can be explored in the field of drug design and development.

DISCOVERY AND FURTHER HISTORY

Herbal medicine and its preparations have been widely used in developing and developed countries for thousands of years in the primary health care of society and community. They are in high demand due to their safety and efficacy with few side effects or unfavorable consequences. India is regarded as a well-documented and well-practiced knowledge country for traditional herbal medicine. The Indian medical systems are mostly Ayurveda, Siddha, and Unani. Traditional herbal remedies are often spontaneously occurring and generated from plants, with little or no industrial methodology required in their ultimate manufacturing, and are used to cure illness, diseases, or disorders in local or regional healing practices. Many herbal medications and phytomedicines are put into the market for community primary health care without any necessary safety or toxicological evaluation of quality control standards pertaining to pharmacological effect. Many countries dealing with the manufacturing and production of herbal medicines lack effective machinery, laboratories for effective quality control testing to govern manufacturing methods and quality standards of herbal preparations. Flavonoids are a type of polyphenolic chemical that is found throughout the plant kingdom. Flavonoids, as a significant active ingredient, have the greatest variety of pharmacological actions. The main flavonoid components, such as quercetin (Fig. 31.1) Quercetin is a flavonoid chemically known as 5.7,3-4 tetra hydroxy flavanol, which has significant pharmacological effects such as hepatoprotective activity, antispasmodic activity, anti-inflammatory activity, and so on. And it primarily refers to polyphenolic derivatives [1-4].

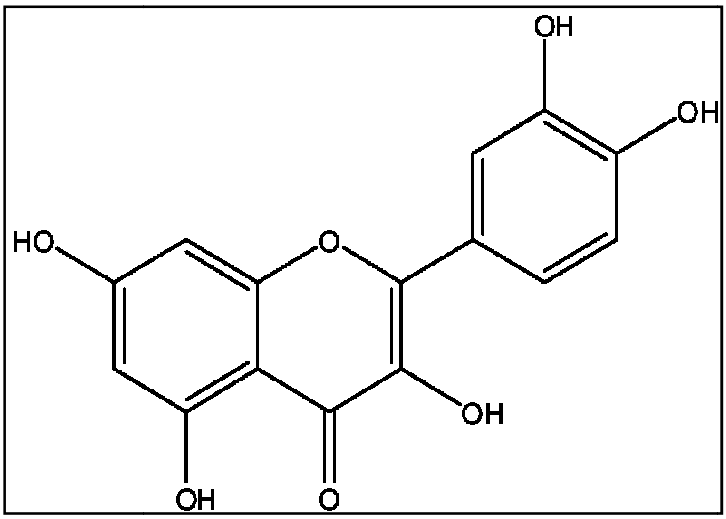


Fig. 31.1 Chemical Structure of Quercetin

Quercetin is a distinct bioflavonoid that has been widely researched by scientists over the last 30 years. Albert Szent Gyorgyi, a Nobel Prize recipient, discovered bioflavonoids in 1930. Flavonoids are natural compounds with varying phenolic structures that can be found in fruits, vegetables, grains, bark roots, stems, flowers, tea, and wine. Long before flavonoids were identified as the potent chemicals, these natural products were known for their health benefits. More than 4000 flavonoids have been found, many of which are responsible for the colorful flowers, fruits, and leaves. These natural ingredients were known for their health advantages even

before flavonoids were identified as the powerful molecules that they are. There are about 4000 flavonoids discovered, many of which are responsible for the colorful flowers, fruits, and leaves. Flavonoids exist in the form of aglycones, glycosides, and methylated derivatives. The flavonoid aglycone is made up of a benzene ring (A) condensed with a six-membered ring (C), which has a phenyl ring (B) as a substituent in the 2-position. Flavonoids are classified into different classes based on their chemical structures. A-pyrone (flavonols and flavonoids) or its dihydro derivative (flavanols and flavanones) is a six-member ring condensed with the benzene ring. The flavonoid class is divided into flavonoids (2-position) and isoflavonoids (3-position) based on the position of the benzenoid substituent. The hydroxyl group, the 3-position, and the C2-C3 double bonds distinguish flavonoids from flavonoids. Flavonoids are frequently hydroxylated at positions 3', 5', 4', and 5'. The alcohol group's methyl ethers and acetyl esters are known to exist in nature. The glycosidic bond is generally produced in positions 3 or 7, and the carbohydrate can be L-rhamnose, D-glucose, glucose-rhamnose, galactose, or arabinose. Flavonoids are classified into seven major categories. Quercetin, one of the most well-known flavonoids, belongs to this class. Onions, broccoli, apples, and berries are high in quercetin. The second type of flavanone is found primarily in citrus fruits. Naringenin is an example of a Flavonoid in this group. Catechin-containing flavonoids are mostly found in green and black tea, as well as red wine, whereas anthocyanins are found in strawberries, other berries, grapes, wines, and tea. The flavonoid content of various meals. Quercetin, the most prevalent flavonoid (the word originates from the Latin -quercetum, which means oak forest, quercus oak), is made up of three rings and five hydroxyl groups. Quercetin belongs to the flavonole class of flavonoids and serves as the foundation for many other flavonoids, including citrus flavonoids such as rutin, hesperidina, Naringenin, and tangeritin. It is found in rinds and barks across the plant kingdom. Quercetin is an aglycon or aglycone that lacks a carbohydrate component in its structure. Quercetin is commonly found in plants in the form of glycine or carbohydrate conjugates. Rutin and thujin are examples of quercetin glycine conjugates. Quercetin-3-rutinoside is another name for rutin.Thujin's other names include quercitrin, Quercetin-3-L-rhamnoside, and 3-rhamnosyl quercetin. Onions contain Quercetin and carbohydrate isorhamnetin conjugates such as Quercetin-3-4'-di-o-beta glucoside, isorhamnetin-4'-o-beta glucoside, and Quercetin-4'-o-beta glucoside. [5-12]

PHYSICOCHEMICAL PROPERTIES

The physicochemical properties of Quercetin which were obtained as computed and experimental were presented in Table 31.1 and Table 31.2 respectively. [13]

Table 31.1 Computed Properties Quercetin

Sl. No.	Property	Property Value
1	Molecular Weight	302.23 g/mol
2	XLogP3	1.5
3	Hydrogen Bond Donor Count	5
4	Hydrogen Bond Acceptor Count	7
5	Rotatable Bond Count	1
6	Exact Mass	302.04 g/mol
7	Monoisotopic Mass	302.04 g/mol
8	Topological Polar Surface Area	127Å²
9	Heavy Atom Count	22
10	Formal Charge	0
11	Complexity	488
12	Isotope Atom Count	0
13	Defined Atom Stereocenter Count	0
14	Undefined Atom Stereocenter Count	0
15	Defined Bond Stereocenter Count	0
16	Undefined Bond Stereocenter Count	0
17	Covalently-Bonded Unit Count	1

Table 31.2 Experimental Properties

Sl. No.	Properties	Description
1	Physical Description	Yellow needles or yellow powder.
2	Color / Form	Yellow needles
3	Boiling Point	Sublimes
4	Melting Point	316.5°C
5	Vapor Pressure	2.81X10-14 mm Hg at 25 °C
6	LogP: log Kow	1.48
7	Henry's Law Constant	6.60X10-21 atm-cu m/mol at 25 °C
8	Optical Rotation	Specific optical rotation: -83 deg at 20 °C/D
	UV max	259, 364 nm
9	Decomposition	When heated to decomposition it emits acrid smoke and irritating fumes.
10	Solubility	Very soluble in ether, methanol Soluble in ethanol, acetone, pyridine, acetic acid
11	Dissociation Constants	pKa1 = 7.17 (Phenol) pKa2 = 8.26 (Phenol)

PHARMACOKINETIC PROPERTIES

The metabolism and pharmacokinetics of flavonoids have been active study topics during the last decade. Approximately 100 research papers have been published to date on the pharmacokinetics of specific flavonoids in healthy volunteers. The data show significant disparities across different types of dietary flavonoids, indicating that the most abundant flavonoids in the diet do not always yield the largest concentration of flavonoids or their metabolites in vivo. The absorption rate in the small intestine ranges from 0 to 60% of the dose, while the elimination half-life (T1/2) ranges from 2 to 28 hours. Quercetin is widely thought to be poorly absorbed. The small intestine absorbs around 25% of a quercetin injection dosage. Although a recent study by Hollman et al concludes that humans absorb appreciable amounts of quercetin, it contradicts the assumption. However, it is found in human plasma as conjugates with glucuronic acid, sulfate or methyl groups, with no significant amounts of free quercetin. Quercetin was found to reach 0.1-10 µmol/lit (micromole per liter) in the circulation. The concentration of quercetin was mainly due to the presence of quercetin metabolites rather than its aglycon as recently revised by Murota and Terao. Regarding the pharmacokinetics of quercetin glucosides conjugates; it seems that the main determinant of absorption of these conjugates is the nature of the sugar moiety. For example, quercetin glucoside is absorbed from the small intestine, but quercetin rutinosides is absorbed from the colon after the carbohydrate moiety is removed by bacterial enzymes. In addition to the chemical form of the flavonol, the fat content of the food influences oral bioavailability of quercetin. Lesser et al studied the effect of dietary fat on the oral bioavailability of quercetin. According to them, quercetin bioavailability from each diet was always higher from the glucoside than from the aglycon, but regardless of the chemical form used, quercetin bioavailability was also observed to be higher in the 17% fat diet compared to the 3% fat diet (P 0.05). Bromelain, an enzyme derived from pineapple, has been demonstrated in studies to improve quercetin absorption. Bromelain is a complex molecule that is mostly made up of proteolytic enzymes. Several investigations have demonstrated that bromelain is a fibrinolytic agent. Bromelain has many of the same antihistamine and anti-leukotriene effects as quercetin. They complement each other's properties in this way. Quercetin is delivered to the liver by portal circulation after being absorbed in the small intestine, where it undergoes first pass metabolism. Quercetin and its metabolites are transported throughout the body to numerous tissues. In plasma, quercetin is tightly linked to albumin. Peak plasma levels are reached between 0.7 and 7.0 hours after intake. Quercetin has an elimination half life of about 25 hours. The clearance of quercetin was considerably delayed following its use in fat-enriched meals (P 0.05). [14,15]

MECHANISM OF WORKING

The quercetin is act by various mechanism actions and hence it can be used as a versatile plant derived drug with multiple pharmacological effects. Few important mechanisms of action of quercetin are discussed below:

Anti-oxidative action

The potential of Quercetin to act as an antioxidant is its most well-known feature. Quercetin appears to be one of the most effective flavonoids for shielding the body from reactive oxygen species, which are formed during normal oxygen metabolism or are triggered by external injury. Lipid peroxidation appears to be one of the most fundamental mechanisms and sequences of events by which free radicals interfere with cellular functioning, finally leading to cell death. Living organisms have created antioxidant defense systems to prevent cellular death caused by reactive oxygen species. These include enzymatic and non-enzymatic antioxidants that keep ROS/RNS levels in check and repair oxidative cellular damage. The key enzymes directly involved in superoxide dismutase, catalase, and glutathione peroxidase neutralization.

Direct radical scavenging effect

Free radical generation in animal cells can be accidental or intentional. With the growing acknowledgement of free radicals as widespread and significant metabolic intermediates, they have been linked to a wide range of human disorders. Quercetin has been found to protect against reperfusion ischemia tissue injury by functioning as a free radical scavenger. Quercetin protects tissue from free radical damage in a variety of ways. The direct scavenging of free radicals is one method. Flavonoids, notably Quercetin, can prevent LDL oxidation in vitro by scavenging free radicals. This activity helps to prevent atherosclerosis.

Inducible nitric oxide synthase Inhibitory action

By interfering with inducible nitric oxide synthase activity, quercetin reduces ischemia-reperfusion damage. Endothelial cells and macrophages are two examples of cells that create nitric oxide. Although the early release of nitric oxide via constitutive nitric oxide synthase activity is vital in maintaining blood channel dilatation, the much higher concentration of nitric oxide produced by inducible nitric oxide synthase in macrophages might cause oxidative damage. Because quercetin scavenges free radicals, it can no longer react with nitric oxide, resulting in reduced damage. Nitric oxide, interestingly, can be regarded as a radical in and of itself, and it can be directly scavenged by Flavonoids.

Xanthine oxidase inhibitory action

The xanthine oxidase pathway has been implicated as a key mechanism in tissue oxidative damage, particularly following ischemia-reperfusion. The enzymes xanthine dehydrogenase and xanthine oxidase are both involved in the conversion of xanthine to uric acid. The enzyme xanthine dehydrogenase is present in healthy settings, but its configuration changes to xanthine oxidase during oxidative stress and ischemia situations. Quercetin appears to suppress xanthine oxidase activity, resulting in less oxidative damage.

Decreasing Leukocyte immobilization

Another significant mechanism responsible for the generation of oxygen-derived free radicals, as well as the release of cytotoxic oxidants and inflammatory mediators and additional activation of the complement system, is the immobility and solid adhesions of leukocytes to the endothelium wall. Leukocytes travel freely over the endothelium walls under normal conditions. Oral administration of purified micronized flavonoids fraction has been shown to reduce the number of immobilized leukocytes after reperfusion, which may be connected to its anti-inflammatory activity.

Modulation of gene expression

Recent research suggests that Quercetin's radical scavenger property is unlikely to be the sole explanation for its neuroprotective capacity, and that a wide range of cellular signaling processes may easily explain their biological effects.

Other Mechanisms

Much recent attention has been paid to Quercetin's ability to interact with intracellular signaling pathways such as the mitogen-activated protein kinase cascade. Quercetin's substantial neurotoxic potential in primary cortical neurons may be mediated by particular and sensitive interactions within neuronal mitogen-activated protein kinase and Akt/protein kinase B (PKB) signaling cascades, both of which have been linked to neuronal apoptosis. Quercetin inhibited both Akt/PKB and ERK phosphorylation, resulting in decreased BAD phosphorylation and significant caspase-3 activation.

Tumor necrosis factor alpha (TNF-α): In a dose-dependent manner, quercetin suppressed TNF- production and gene expression. A reduction in endogenous TNF- generation in the presence of quercetin suggests that flavonoids can modify the immune response and have an anti-inflammatory effect. TNF-, in addition to its well-known proinflammatory activity, has complex effects on immune cell proliferation, differentiation, and death.

Interaction with other enzyme systems

Arachidonic acid is an important substrate for thromboxane, inflammatory prostaglandins, and leukotrienes. Furthermore, Quercetin inhibits the enzymes cyclooxygenase and lipoxygenase, which catalyze the conversion of arachidonic acid to its metabolites. Reducing levels of these metabolites, as well as histamine levels, aids in the maintenance of normal body tissue and structure comfort. Quercetin has also been demonstrated to inhibit adhesion molecule action on endothelial cells. Quercetin also chelates transition metal ions like iron, which can cause the generation of oxygen free radicals. Another form of protection is the direct suppression of lipid peroxidation. Calmodulin, a calcium regulating protein, interacts with quercetin. Calmodulin transfers calcium ions across cell membranes, triggering a variety of biological processes. Quercetin appears to have calmodulin antagonist properties. Quercetin acts as a membrane stabilizer at the cell membrane level via this method. Quercetin inhibits calmodulin-dependent enzymes found in the cell membrane, such as ATPases and phospholipases, affecting membrane permeability. Quercetin influences other calmodulin-dependent enzymes that regulate cellular processes such as histamine production from mast cells. Several studies have shown that Quercetin can inhibit histamine release from mast cells in various organs as well as basophils. Quercetin's enzyme inhibition extends to phospholipases, which catalyze the release of arachidonic acid from phospholipids contained in cell membranes.

METHODS OF SYNTHESIS

The first effective synthesis of quercetin was reported in 1962, when 2-methoxyacetyl phloroglucinol was treated in triethylamine with O-benzyl vanillic acid anhydride and subsequently with potassium hydroxide to create 5, 7-dihydroxy-4'-benzyloxy-3, 3'-dimethoxyflavone. To create quercetin, the benzyl ether was cleaved using acetic acid-hydrochloric acid, and the methyl ethers were cleaved with hydriodic acid.

MEDICINAL USES

Quercetin offers a variety of potential therapeutic uses primarily in the prevention and the treatment of the conditions listed below.

- Quercetin is used in treatment of allergies, asthma, hay fever and hives
- Quercetin is used in the management of heart diseases.
- It is used to reduce the high cholesterol level
- It is used in the treatment and management of hypertension
- It is used to treat the interstitial cystitis
- It is used in effective treatment of prostatitis
- It is used to manage Rheumatoid arthritis
- It has showed good medicinal property in treatment and prevention of variety of Cancers
- It exerts good antibacterial activity against different types of bacteria.
- It has good medicinal value in managing the coronary heart diseases
- Quercetin is well known for the management of Diabetic complications
- It is used to treat the eye disorders
- It can be used to treat the Gout
- It has very good therapeutic effect in Neurodegenerative disorders
- It is used to treat the Osteoporosis
- It has showed the effect in treatment of Peptic Ulcer
- It is used in treatment of Prostatitis
- It is also used as antiviral agent in treatment of viral infections

In order to support the above medicinal properties of Quercetin several studies have been performed and few of such investigations are discussed as below:

- **Allergies, asthma, hay fever and hives:** In-vitro studies have shown that quercetin inhibits the release of histamines, which are molecules that induce allergic reactions. As a result, experts believe quercetin may help lessen allergy symptoms such as runny nose, watery eyes, hives, and swelling of the face and lips. However, there is no proof that it works in humans.
- **Heart disease:** The flavonoids quercetin, resveratrol, and catechins (all found in high concentrations in red wine) may help reduce the risk of atherosclerosis, plaque buildup in arteries that can lead to heart attack or stroke, according to test tube, animal, and some population-based research. These nutrients appear to protect against LDL (bad) cholesterol damage and may help avoid heart disease death. However, the majority of human investigations have focused on flavonoids in the diet rather than as supplements. Animal studies have employed extraordinarily high doses of flavonoids (much more than a supplement could provide). More human research is needed to determine whether flavonoid supplements are beneficial.
- **High cholesterol:** Quercetin reduces LDL cholesterol damage in test tubes, and population studies demonstrate that persons who eat flavonoid-rich diets have lower cholesterol. According to one study, those who took quercetin and an alcohol-free red wine extract (which contains quercetin) had decreased LDL cholesterol damage. Another study discovered that quercetin lowered LDL

levels in overweight people at high risk of heart disease. More research is needed to determine whether taking a quercetin supplement has the same impact.

- **Hypertension:** According to research, quercetin supplementation lowers blood pressure in hypertensive persons.
- **Interstitial cystitis:** Flavonoids may help persons with interstitial cystitis, according to two small trials. People with this disorder have bladder pain similar to that of a bladder infection, as well as an urgent need to urinate. Those who took a quercetin supplement appeared to have fewer symptoms in both investigations. However, other flavonoids were included in the trials. As a result, it is unknown which flavonoid provides the most advantages. There is a need for more and better-designed investigations.
- **Prostatitis:** Preliminary research suggests that quercetin may alleviate symptoms of prostatitis, or prostate inflammation. In one tiny trial, men who took quercetin had less symptoms than men who received a placebo. More investigation is required.
- **Rheumatoid arthritis (RA):** There have been reports of persons with RA experiencing reduced symptoms after switching to a vegan diet rich in uncooked berries, fruits, vegetables, nuts, roots, seeds, and sprouts containing quercetin and other antioxidants. However, there is no indication that the beneficial effects were caused by antioxidants, and there is no proof that quercetin supplements assist treat RA.
- **Cancer:** Quercetin and other flavonoids found in fruits and vegetables have long been thought to be beneficial in cancer prevention. People who eat more fruits and vegetables have a lower risk of developing some types of cancer. Flavonoids have anti-cancer potential, according to animal and test tube research. In these research, quercetin and other flavonoids were demonstrated to suppress the growth of cancer cells from breast, colon, prostate, ovarian, endometrial, and lung cancers. O is linked to a decreased incidence of lung cancer. The link was significantly stronger among patients who smoked more than 20 cigarettes per day, while a third study found that quercetin suppresses tumor growth in leukemia cells in the lab. More investigation is required.
- **Antibacterial activity:** Quercetin appears to have antibacterial activity against nearly all of the bacteria strains known to cause respiratory, gastrointestinal, skin, and urinary diseases.
- **Arthritis:** Quercetin suppresses the actions of both cyclo-oxygenase and lipo-oxygenase, reducing the generation of inflammatory mediators. Furthermore, there have been instances of persons with rheumatoid arthritis experiencing an improvement in their symptoms after switching from a standard western diet to a vegan diet rich in raw berries, fruits, and vegetables, which contain, among other antioxidants, quercetin.
- **Coronary Heart Diseases:** Consumption of the antioxidant quercetin protects against coronary heart disease (CHD) caused by oxidized LDL. According to Hertog et al, frequent consumption of flavonoids in food may reduce the incidence of CHD mortality in elderly men. In addition, a Japanese study found an inverse relationship between quercetin consumption and total plasma cholesterol content. Quercetin has also been demonstrated to be an efficient platelet aggregation inhibitor in dogs and monkeys. The predominant antiplatelet aggregating effect is due to thromboxane A2 inhibition. Quercetin suppresses aortic smooth muscle cell proliferation and migration, as well as platelet aggregation and mitogen-activated protein kinase activation. These findings offer new information and support for the possible use of quercetin in the prevention of cardiovascular disease.
- **Diabetic complications:** Quercetin has been discovered to be an inhibitor of the enzyme aldose reductase, which is involved in the body's conversion of glucose (sugar) to sorbitol (a sugar alcohol). Because of sorbitol buildup in the body, people with diabetes acquire secondary issues such as neuropathy, retinopathy, diabetic cataracts, and nephropathy. Quercetin may thus be advantageous in the nutritional treatment of diabetes, although clinical trials are needed to confirm the results seen in non-human tests.
- **Eye disorders:** Free radicals are thought to play a role in the development of certain diseases such as cataracts and macular degeneration. By neutralizing free radicals, quercetin prevents and heals various eye diseases. In a study of 3,072 persons with macular degeneration symptoms, moderate red wine drinking (a source of quercetin) provided some protection against the disease's development and progression. Dark berries have been shown to help prevent macular degeneration when consumed on a regular basis.
- **Gout:** Quercetin's xanthine oxidase inhibiting nature reduces the synthesis of uric acid, alleviating gout symptoms.
- **Neurodegenerative disorders:** Quercetin appears to maintain brain functioning by preventing the production of fibrillated amyloid-beta, the senile plaque present in Alzheimer's disease brain. Quercetin has the potential to cure neuroleptic-induced extrapyramidal adverse effects, such as those caused by haloperidol. Quercetin is also a potent antioxidant that has been shown to protect brain cells from harm.
- **Osteoporosis:** The bone mineral density of elderly women who drank tea and those who did not was compared. Women who drank tea (quercetin) in the study had higher bone mineral density values than those who did not. Quercetin in tea may be responsible for osteoporosis prevention.
- **Peptic Ulcer:** Quercetin appears to be quite significant in the prevention and treatment of peptic ulcers. It works as a gastroprotective agent by increasing mucus secretion. Many peptic ulcers appear to be caused by the pathogenic bacterium Helicobacter pylori. In-vitro investigations have revealed that quercetin inhibits the growth of this bacterium.
- **Prostatitis:** Quercetin was proven to be beneficial in category III chronic prostatitis (nonbacterial chronic prostatitis with prostatodynia) in a prospective double-blind placebo controlled research. For one month, thirty males with this disease were given either a placebo or 500 milligrams of quercetin twice daily. The National Institute of Health Chronic Prostatitis score showed significant improvement in the treated group. In a subsequent unblinded open study, more males were given the same quantity of quercetin for one month, but this time the quercetin was mixed with bromelain and papain, which may improve absorption. In this study, 82% of participants improved by at least 25%.
- **Viral infections:** In a study conducted by Wang et al., the antiviral action of flavonoid was demonstrated. Flavonoids have been reported to influence viruses such as herpes simplex virus, respiratory syncytial virus, and adenovirus. Quercetin has been shown to have anti-infective and anti-replicative properties. The vast majority of investigations were conducted in vitro, and nothing is known regarding the antiviral activity of flavonoids in vivo. Flavonoids in their glycon form appear to have a greater inhibitory

effect on rotavirus infectivity than flavonoids in their aglycon form. Because of the global expansion of HIV, research into the antiviral effect of flavonoids has mostly focused on HIV since the 1980s. Flavonoids have been discovered and developed as anti-HIV drugs throughout the last two decades. The majority of the research focused on reverse transcriptase or RNA directed DNA polymerase inhibitory activity, however anti integrase and antiprotease activities were also described. Flavonoids have primarily been investigated in vitro; hence no obvious contribution of flavonoids to the treatment of HIV-infected patients has yet been demonstrated.

ADVERSE EFFECTS

The following side effects have been documented with oral quercetin:

- Nausea and other digestive disorders.
- A few people have reported headaches and slight tingling in their limbs.
- Nausea, vomiting, diaphoresis, flushing, and dyspnea have been recorded following intravenous quercetin administration.
- When taken orally for a short period of time, quercetin may be safe for most people. • Quercetin has been administered safely for 12 weeks at doses of up to 1 gram daily. Long-term use or higher doses are not known to be safe.
- When applied to the skin: There is insufficient credible evidence to determine whether quercetin is safe or what the potential side effects are.

Safety Profile

A examination of the whole body of available data on quercetin as reported in multiple published publications suggests that, while mutagenic in vitro, quercetin is not carcinogenic in the body. A review of quercetin safety based on previous animal toxicity studies, such as Formica and Regelson, Stavric, Stoewsand, and recently Okamoto, determined that orally administered quercetin is unlikely to induce any harmful effects, albeit particular dose levels were not indicated.

TREATMENT OF OVERDOSE

In the short term, quercetin may be safe for most people when taken orally. Quercetin has been used successfully for 12 weeks in doses of up to 1 gram per day. Long-term use or higher doses are not known to be safe. When applied to the skin: There is insufficient trustworthy evidence to determine whether quercetin is safe or what the probable side effects are. Pregnancy and breast-feeding: Avoid utilizing to be on the safe side. Renal problems: Quercetin may worsen renal problems. If you have kidney problems, stay away from quercetin.

CONTRAINDICATIONS

Quercetin has no known contraindications. In test tube investigations, quercetin has been demonstrated to cause chromosomal changes in certain bacteria. However, the importance of this discovery for people is unclear. Quercetin should be avoided by pregnant women and nursing mothers due to a lack of long-term safety data.

INTERACTIONS

Quercetin shows interaction with following drugs:

- Felodipine: In test tube research, quercetin (present in grapefruit juice, tea, onions, and other foods) was shown to block enzymes responsible for breaking down Felodipine into inactive forms. This combination may result in elevated felodipine blood levels, which may cause undesired side effects.
- Estrogens: Grapefruit juice has been demonstrated in studies to considerably raise estrogen levels in the blood. Quercetin is one of the flavonoids found in grapefruit juice. In a test tube investigation, quercetin was discovered to alter estrogen metabolism in human liver cells, increasing estradiol levels while decreasing other types of estrogen.
- Grapefruit juice induces a significant increase in cyclosporine blood levels when compared to cyclosporine with water in a randomized investigation of nine adults with cyclosporine-treated auto-immune disorders.
- Quinolones: Quercetin binds to the DNA gyrase site in bacteria in vitro. As a result, it may act as a competitive inhibitor to quinolones, which also bind to the same location.
- Cisplatin: Because of the possible risk of genotoxicity in normal tissues, patients taking cisplatin should avoid taking quercetin supplements.
- Doxorubicin: Animal and test tube research indicate that quercetin may boost the impact of doxorubicin.
- Digoxin: Treatment with both Digoxin and Quercetin may result in high levels of digoxin in the blood, which may produce more digoxin side effects than usual. This connection has been observed in animals, but its impact on humans is unknown.

Dosing

Adults have typically taken 250-1000 mg of quercetin orally daily for up to 12 weeks. Speak with a healthcare provider to determine the appropriate dose for a specific disease.

NOVEL MARKETED PREPARATIONS

Quercetin shows interaction with following drugs:

- Felodipine: In test tube research, quercetin (present in grapefruit juice, tea, onions, and other foods) was shown to block enzymes responsible for breaking down Felodipine into inactive forms. This combination may result in elevated felodipine blood levels, which may cause undesired side effects.
- Estrogens: Grapefruit juice has been demonstrated in studies to considerably raise estrogen levels in the blood. Quercetin is one of the flavonoids found in grapefruit juice. In a test tube investigation, quercetin was discovered to alter estrogen metabolism in human liver cells, increasing estradiol levels while decreasing other types of estrogen.
- Grapefruit juice induces a significant increase in cyclosporine blood levels when compared to cyclosporine with water in a randomized investigation of nine adults with cyclosporine-treated auto-immune disorders.
- Quinolones: Quercetin binds to the DNA gyrase site in bacteria in vitro. As a result, it may act as a competitive inhibitor to quinolones, which also bind to the same location.

- Cisplatin: Because of the possible risk of genotoxicity in normal tissues, patients taking cisplatin should avoid taking quercetin supplements.
- Doxorubicin: Animal and test tube research indicate that quercetin may boost the impact of doxorubicin.
- Digoxin: Treatment with both Digoxin and Quercetin may result in high levels of digoxin in the blood, which may produce more digoxin side effects than usual. This connection has been observed in animals, but its impact on humans is unknown.

Dosing

Adults have typically taken 250-1000 mg of quercetin orally daily for up to 12 weeks. Speak with a healthcare provider to determine the appropriate dose for a specific disease.

CONVENTIONAL MARKETED PREPARATIONS

Some of the important formulations of quercetin are listed in Table 31.3 as shown below:

Table 31.3 Marketed Formulations of Quercetin

Sl. No.	Type of Formulations	Brand Name	Manufacturers
1	Quercetin Capsules	Covilife QTN	Bombay Medicos
2	Quercetin Tablets	Celizaren	Vallabh Enterprise

Fruit and vegetable consumption can provide 15 to 40mg of quercetin per day on average. Increasing quercetin intake for general health benefits is as simple as eating more veggies and fruit. However, because most people are unable to receive an appropriate intake of flavonoids from food sources, extra quercetin can be gained through dietary supplements. Higher doses of quercetin are typically prescribed for therapeutic objectives such as allergy control, anti-inflammatory treatment, and disease treatment. Dosages for therapeutic purposes might range from 250 to 500 mg three times per day.

NOVEL MARKETED PREPARATIONS

The novel marketed formulations of Quercetin are listed as below:

- Quercetin supplements as pills and capsules in combination with bromelain for anti-inflammatories.
- Quercetin rich extracts loaded with grapeseed, bilberry, *Ginkgo biloba*, and green tea are available as novel formulation.
- A novel water-soluble quercetin formulation such as hesperidin-methyl-chalcone (HMC) or quercetin-chalcone is also available in the market.
- Nanoliposomes of Quercetin.

PATENTS

Few of the important patent's publications on Quercetin are listed in Table 31.4.

Table 31.4 Patent Publications on Quercetin

Sl. No.	Patent Publication Number	Title of the Patented Work
1	KR-20220167790-A	Novel Aldehyde Dehydrogenase Composition for Improving Behavior and Motor Function
2	CN-115399343-A	A plant compound antibacterial agent containing peony extract and its preparation method and application
3	CN-115399343-B	A plant compound antibacterial agent containing peony extract and its preparation method and application
4	CN-115606668-A	A kind of anti-aging food-grade functional tea and preparation method thereof
5	CN-115414301-A	A plant anti-dandruff composition and its preparation method and application

COMPUTER AIDED DRUG DESIGN SCREENING STUDIES OF QUERCETIN

Computational Drug Likeness Prediction of Quercetin

MolSoft web servers (https://molsoft.com/mprop/) were used to forecast the drug-like characteristics of phytocompounds. Lipinski's rule of five was used to compute drug-like qualities, which states that molecules should have a molecular weight of 500, a C logP of 5, less than 10 hydrogen bond acceptors, and less than 5 hydrogen bond donors. To anticipate drug-like features, the canonical simplified molecular line-entry systems were obtained from PubChem and entered into the MolSoft online server. The results shown in Table 31.5 show that the majority of the selected plant constituents had acceptable values for most of the parameters such as compound molecular weight (500 Daltons), number of hydrogen bond acceptors (10) and number of hydrogen bond donors (5) and polar surface areas (90-140) within recommended ranges.

Table 31.5 Physicochemical properties & drug likeness data of Quercetin

Drug	MW	HBA	HBD	Log P	PSA	RO5	Drug likeness
	<500	**≤ 10**	**≤ 5**	**≤ 5**	**90-140 (A^2)**	**≤ 1**	**>0**
Quercetin	302.04	7	5	1.19	114.055	0	0.52

In-silico ADMET Properties of Quercetin

Quercetin ADME parameters were determined using Schrodinger's software using the Maestro 12.4 interface. SDF files were loaded into the software, and an ADME analysis was carried out with the help of LigPrep and QikProp. ADME (Absorption, Distribution, Metabolism, and Elimination) investigations look into how a chemical (a medicinal ingredient) is metabolized by a living organism. Various ADME characteristics of the tested medications were estimated in this study to better understand the impact of physicochemical and pharmacokinetic qualities on bioavailability patterns in the human body. The ADMET characteristics of Quercetin are represented in Table 31.6.

Table 31.6 Pharmacokinetic parameters of Quercetin

CNS	mol MW	dipole	volume	QPlog PC16	QPlog Poct	QPlog Pw	QPlog Po/w	QPlog S	CIQP log S	QPlog HERG	QPP Caco	QPlog BB	QPPM DCK	QPlog Kp	QPlog Khsa	%HOA	PSA	RO5	RO3
0	154	1.85	694	5.46	7.21	3.07	2.64	-2.3	-1.6	-3.4	4177	-0.1	2319	-1.5	-0.1	100	21	0	0

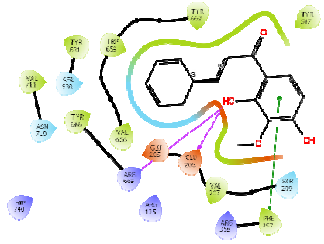
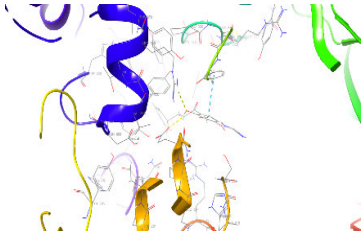
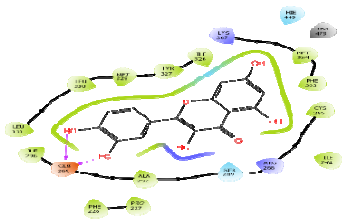
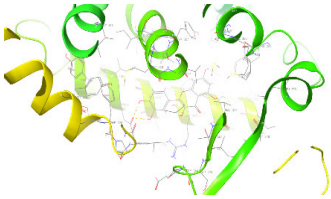
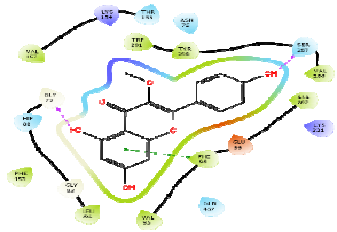
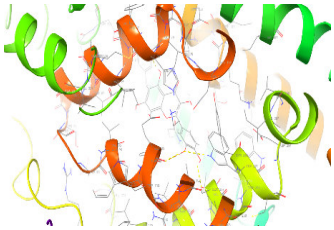
Molecular Docking Studies of Quercetin with Diabetes Mellitus Targets

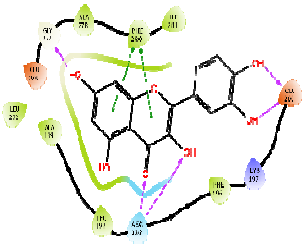
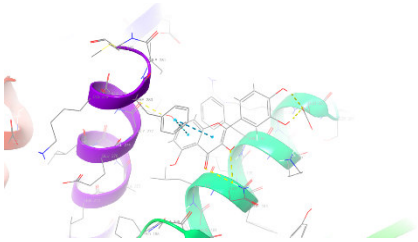
Docking is a method that predicts the preferred orientation and pose of a ligand molecule within a receptor, and then uses scoring functions to offer information on the interactions, binding affinity, or strength of association between the ligand and the receptor. The docking algorithm arranges molecules in complementary ways. Protein Data Bank (https://www.rcsb.org/) was used to retrieve the three-dimensional protein structures of chosen protein receptors found in Diabetes mellitus targets. Docking experiments were carried out using the Glide Module of Schrodinger's Molecular Modelling software (Schrodinger Inc., USA, 2020-2). Target proteins for inverse docking included DPP4: dipeptidyl peptidase-4 [PDB ID: 6B1E], PPAR-gamma: peroxisome proliferator activated receptor [PDB ID: 2P4Y], SGLT2: sodium glucose cotransporter 2 [PDB ID: 7VSI], and PTP1B: protein tyrosine phosphatase 1B [PDB ID: 1T49]. Each of these protein targets was docked by Quercetin. All docking interactions (2D and 3D) were assessed using Schrodinger's Maestro graphical interface. Table 31.7 displays the docking results of selected phytochemicals. Table 31.8 displays 2D and 3D images of ligand target and amino acid interaction of selected elements against various targets. **[16-20]**

Table 31.7 Docking score and glide energy of Quercetin against various targets of Diabetes Mellitus

Drug	Docking Score (kcal/mol)				Glide Energy (kcal/mol)			
	DPP4	PPAR		DPP4	PPAR		DPP4	PPAR
Quercetin	-4.906	-8.118	-8.631	-6.815	-40.744	-47.958	-50.136	-38.27

Table 31.8 2D and 3D Interaction of Quercetin against Diabetes Mellitus targets

Target ID	2D Interaction	3D Interaction
6B1E		
2P4Y		
7VSI		

1T49		

ANALYTICAL STUDIES ON QUERCETIN

Quality assurance and standardization of phytomedicines are critical. Herbal medical formulations have been widely used for primary health care in all developing and developed countries from time immemorial. They are frequently used because they provide numerous additional benefits in terms of safety, efficacy, and few or no side effects. Traditional herbal remedies are made from plants that grow naturally and have medicinal characteristics. These preparations, which do not require extensive industrial methods to manufacture, are currently in high demand for the treatment of a variety of maladies, including serious health issues. India is well-known for its expertise in natural medicine. The Indian systems of medicine are primarily Siddha, Ayurveda, and Unani systems, which serve as the foundations of the Indian traditional system of medicine. Herbal treatments may not necessitate the evaluation of phytochemical toxicity or safety profiles. Many countries are currently engaged in the creation and production of phytomedicines, but they lack effective laboratories and technology for the quality control and standardization of herbal formulations. Quality control is essential for any formulation that is to be marketed since product quality implies the safety and effectiveness of medicines. Quality control is required for all raw materials, excipients, and final goods. The quality of the materials used in the creation of phytomedicines must be monitored before they are employed in the final product. This can be accomplished with the assistance of numerous modern analytical equipment available on the market, which play a vital role in quality control and standardization. These include widely used electrochemical, chromatographic, and spectroscopic approaches. The rapid global market for herbal medicines and phytopharmaceuticals is expanding due to a significant increase in demand. As a result, health organizations, pharmaceutical businesses, and the general public are concerned about the safety and quality of phytopharmaceuticals. There is a high demand for maintaining consistent quality, safety, efficacy, and potency from batch to batch. "Standardization of Phytopharmaceuticals based on Marker" is a widely established method all over the world. The method is based on the examination of phytochemical markers utilizing sophisticated analytical methods and instruments, and it is used to establish quality control data for herbal goods. The idea of standardizing herbal medications using marker-based technology has recently gained traction. Marker chemicals are widely used as analytical instruments to assess the quality of phytopharmaceuticals around the world. 4,5 In general, a marker serves as a reference for standardizing test materials, such as raw materials, extracts, and finished products, and is thus utilized for quality assurance of phytopharmaceuticals in the herbal industry. A marker chemical is a component of a natural medication that has or does not have therapeutic effect. Markers are reference chemicals that are chemically specified phyto-constituents that may or may not have any biological action. Markers are employed to quantify phytopharmaceutical active components. Marker chemicals are secondary metabolites that are extracted and purified.

We have various contemporary devices and analytical procedures that may be used to control the quality of natural medicines or medications. Chromatographic Techniques, Optical Methods or Spectral Techniques, Physical Methods or Thermo-analytical Methods, Electrochemical Techniques, Biological and Microbiological Methods, Titrimetric Methods, Radioactive Methods, and Miscellaneous Methods are the various categories of analytical techniques. Spectrum approaches are now used extensively in the structural characterisation of substances derived from both natural and synthetic sources. Spectroscopic technologies are also utilized to perform quantitative and qualitative analyses on raw materials and pharmaceutical formulations. Spectroscopic techniques are frequently utilized in herbal medicine development for characterization of isolated fractions, identification of bioactive fractions, quantitative quantification of bioactive components, and so on. UV Spectroscopy is mainly used for the qualitative and quantitative analysis of drugs or molecules from natural and synthetic origin. Flavonoids form the major active constituent of plants that belong to a class of polyphenols, which are distributed throughout the plant kingdom. Different flavonoids are well known for their potent therapeutic activities. Few of the important flavonoids like Quercetin, Apigenin, and Rutin are well known for their anti allergic, antithrombotic, antispasmodic, anticancer, hepatoprotective, anti-inflammatory, antiallergic and antioxidant activities.[21-61]

Spectrophotometric Quantification of Quercetin

Literature review on the quality control analysis and standardization of Quercetin in its isolated form, extract or any other herbal or polyherbal preparation was done. Review of scientific publications has been added. A complete and detailed summary of spectroscopic standardization of Quercetin is presented below in Table-**31.9.**

Table 31.9 Spectroscopic standardization of Quercetin in herbal medicines

Sl. No.	Author Name	Title of the Work	Description of Analysis
1	V.C. Yeligar *et al.,*	Development and Validation of UV Spectrophotometric Method for Simultaneous Estimation of Melatonin and Quercetin in Liposome Formulation.	The UV spectra are between 200 and 400 nm. Melatonin (10 µg/ml) and quercetin (10 µg/ml) standard solutions were found to have λmax values of 276 nm and 372 nm, respectively.
2	Singh Upendra *et al.,*	Simultaneous Estimation of Quercetin and Silymarin: Method Development and Validation	The wavelengths of the UV spectrum are 400–200 nm. For quercetin and silymarin, the peak absorption was recorded at 256 nm and 288 nm respectively.
3	Ginpreet Aneja *et al*.,	Simultaneous Estimation of Piperine, Quercetin, and Curcumin in A Mixture using UV-Visible spectrophotometer and Method Validation	The maximum absorbance of quercetin, curcumin, and piperine were determined to be 371.31 nm, 424.68 nm, and 343.76 nm, respectively, in the standard stock solution of 1000 ppm of each medication in methanol and scanned in the range of 200-400 nm.
4	A Viswanath *et al*.,	UV spectrometry method for the estimation of Quercetin from *Ipomoea sepiaria* Koenig.	The standard absorbance of quercetin is 350 nm, acetone extract is 328.2 nm, and methanol extract is 323.4 nm.
5	Marzanna Kurzawa *et al.,*	Determination of Quercetin and Rutin in Selected Herbs and Pharmaceutical Preparations	In methanol and ethanol, respectively, the standard solutions of rutin and quercetin were created. For quercetin and rutin, the absorbance was reported at 425 nm and 362 nm, respectively.

Chromatographic Estimation of Quercetin

Literature review on the quality control analysis and standardization of Quercetin in its isolated form, extract or any other herbal or polyherbal preparation was done. Review of scientific publications has been added. A complete and detailed summary of chromatographic standardization of Quercetin is presented below in Table-**31.10**.

Table 31.10 Chromatographic Standardization of Quercetin in herbal formulations

Sl. No.	Author Name	Title of the Work	Description of Analysis
1	Sunita shailajan *et al.,*	A comparative estimation of quercetin content from Cuscuta reflexa Roxbusing validated HPTLC and HPLC techniques	Using 0.025 M NaH2PO4 and ACN as the mobile phase, using HPLC. The investigation was carried out on a C18 column (150mm4.6mm5m), and peaks at 378 nm were noted.
2	Asma'aAi-Rifai *et al*.,	Analysis of Quercetin and Kaempferol in an Alcoholic Extract of Convolvulus pilosellifolius using HPLC	Peaks at 258 nm were detected using the HPLC technique with an isocratic combination of methanol and water containing 0.1% v/v formic acid (80:20) on a BETASIL C18 column.
3	Lee Fung Ang *et al*.,	HPLC Method for Simultaneous Quantitative Detection of Quercetin and Curcuminoids in Traditional Chinese Medicines	Using a thermo ersil Gold column, a C-18 cartridge guard column, and a mobile phase system of acetonitrile and 2% v/v acetic acid (40:60), at the detection wavelength of 370 nm, quantitative detection of quercetin and curcuminoids (dimethoxy curcumin, bis demethoxy curcumin, and curcumin) in traditional Chinese medicines.
4	Ujjwala supe *et al.,*	Preliminary Phytochemical Analysis and Quantitative Analysis of Quercetin by HPLC of *Momordica Charantia.*	Utilizing Merck C-18 Bondapak, which is kept at a temperature of 27°C, and methanol: ACN: water (60:20:20v/v) as the mobile phase, HPLC was utilized to analyze the sample. The investigation was done for flavonoids, phenols, and quercetin at 260 and 262 nm wavelength.
5	A. Srinivasa Rao *et al*.,	Simultaneous determination of phenolic compounds in Catharanthus roseus leaves by HPLC method.	Maximum absorbance was measured at 254 nm using the HPLC method using the Athena C18 column, phosphate buffer (pH=5.8), and ACN as the mobile phase 55:45 Ratio. Rutin, Quercetin, and Kaempferol

			each had a retention time of 2.403, 6.143, and 8.903 correspondingly.
6	Deepak Mundkinajeddu *et al.,*	Development and Validation of High Performance Liquid Chromatography Method for Simultaneous Estimation of Flavonoid Glycosides in *Withania somnifera* Aerial Parts.	HPLC method by using Phenomenex Luna C18 column and the mobile system consist of potassium dihydrogen orthophosphate dissolved in 900 ml of HPLC grade water to that 0.5ml of orthophosphoric acid added volume made up to 1000 ml, for the estimation of 3 flavonoid glycosides that are quercetin-3-rutinoside-7-O-glucoside, quercetin- 3-O-rutinoside-7-O-glucoside, kaempferol 3-O-rutinoside-7-O-glucoside.
7	Aline Augusti Boligon *et al.,*	Development and Validation of an HPLC-DAD Analysis for Flavonoids in the gel of Scutia buxifolia	They have verified the HPLC by quantifying the quercetin and rutin at a maximum absorbance wavelength of 356 nm using a C18 column and a mixture of Acetonitrile: water (70:30, v/v) as the mobile phase.
8	Gomathy Subramanian *et al.,*	Development and Validation of HPLC Method for the Simultaneous Estimation of Quercetin and Rutin in *Aganosma Dichotoma* [Roth] K. Schum	For the HPLC procedure, a C18 column and mobile phase containing acetonitrile: ammonium acetate (40:60 v/v) at a flow rate of 1 ml/min are used to achieve separation. The 259 nm analysis was seen.
9	Haritha Krishna prasad *et al*.,	Method Development and Validation for the Simultaneous Estimation of Resveratrol and Quercetin in Bulk and Pharmaceutical Dosage Form by RP-HPLC	RP-HPLC method for the estimation is performed by using Sunfire C18 column having Rheodyne injector using Methanol: Water:Formic acid: Triethylamine in the ratio 10:70:15:5 as mobile phase at the wavelength 277nm.resveratrol and Quercetin eluted at retention time 1.24 and 2.14 respectively
10	Vishal Sharad Chaudhari *et al.,*	Analytical method development and validation of reverse-phase high-performance liquid chromatography (RP-HPLC) method for simultaneous quantifications of quercetin and piperine in dual-drug loaded nanostructured lipid carriers	RP-HPLC method for The estimation was carried out by using Hypersil gold C-18 column and mixture of acetonitrile and HPLC grade water (pH 2.6 adjusted with 2%v/v glacial acetic acid) as a mobile phase wavelength 346 nm Quercetin and Piperine is eluted at retention time 2.80 min and 10.36 min respectively.
11	Ashok Kumar *et al.,*	Estimation of Gallic acid, Rutin and Quercetin in *Terminalia chebula* by HPTLC	HPTLC method by using precoated silica gel GF254 as stationary phase and Toluene: acetone: glacial acetic acid (3:2:1 v/v/v/v/v) as mobile phase for of tannins, and ethyl acetate: dichloromethane: formic acid: glacial acetic acid: water (10:2.5:1:1:0.1v/v/v/v/v) the quantification is carried out for Rutin and Quercetin at 366 nm and for gallic acid at 254 nm densitometrically and Rf value of gallic acid, rutin, and quercetin are 0.30, 0.13, 0.93 respectively.
12	Sachin U. Rakesh *et al*.,	HPTLC Method For Quantitative determination of Quercetin in Hydroalcoholic Extract Of Dried Flower of *Nymphaea Stellata* Willd.	In the HPTLC method the detection and quantification were carried out by silica gel 60 F254 plates and Toluene: ethyl acetate: formic acid in the ratio 5:4:0.2 (v/v/v) as mobile phase. At 380 nm a sharp and well-defined peak is found at Rf=0.29.
13	Ansul Shakya *et al*.,	A Rapid High-Performance Thin-Layer Chromatographic Method to Estimate Quercetin in *Benincasa casahispida* (Thunb.) Cogn. Fruit Pulp	HPTLC for the estimation using alumina plates with silica gel 60 F254 were used with Toluene: ethyl acetate: formic acid (5:4:0.2v/v) as mobile phase at absorbance wavelength 262 nm the Rf value of Quercetin found Rf=0.392
14	Barik Laxmi Dhar *et al*.,	HPTLC method for Quantitative estimation of Quercetin in a polyherbal compound for urolithiasis	By HPTLC method, extract chromatogram med on silica gel 60 F 254 aluminium plates and mixture of chloroform: methanol: formic acid used as mobile phase in the ratio 7.5:1.5:1 v/v/v. The Rf value was 0.51 analyzed at wavelength of 254nm
15	Bindu A R *et al.,*	High Performance Thin Layer Chromatographic Method for Quantitative Determination of Quercetin in Tender Leaves of Psidium guajava.	HPTLC method carried out on silica gel 60 F 254 TLC plates using toluene: acetone: formic acid (30:10:5) as mobile phase. The detection and quantification done at 364nm and Rf value of acetone extract was 0.45.
16	Vaidevi Sethuram *et al.,*	Combinatorial analysis of quercetin and resveratrol by HPTLC in *Sesbania grandiflora* /phyto based nanoformulations.	HPTLC method In this investigation separation is achieved by using a mobile phase system of toluene: chloroform: ethyl acetate: formic acid (3:2:4.9:0.1 % v/v). The densitometric scanning at 280 nm was

			performed and Rf value was 0.40 (Quercetin) and 0.50 (Resveratrol)
17	Mangesh S. Kharate *et al.,*	Estimation of Quercetin from Crude Leaf Extract, *Mimusops Elengi* L. By HPTLC	Toluene, ethyl acetate, and formic acid were combined in a 5:4:1 ratio as the mobile phase for the detection of quercetin on precoated silica gel plates F 254. There was detection and quantification at 365 nm.
18	V Patil *et al.,*	Recent progress in simultaneous estimation of rutin, quercetin and liquiritin in *Cocculus hirsutus* by HPTLC	The silica gel 60 F 254 was used as the mobile phase system for the simultaneous estimation together with a mixture of n-butanol, acetic acid, water, and formic acid (7:1:1:0.25). At a wavelength of 254 nm, the Rf values for rutin, quercetin, and liquidity were, respectively, 0.047, 0.063, and 0.82.Toluene, ethyl acetate, and formic acid were combined in a 5:4:1 ratio as the mobile phase for the detection of quercetin on precoated silica gel plates F 254. There was detection and quantification at 365 nm.
19	Omi Laila *et al.,*	Development and Validation of HPTLC Method for Simultaneous Estimation of Diosgenin and Quercetin in Fenugreek Seeds (*Trigonella foenum-graceum)*	Precoated aluminium plates with silica gel G60F254 and a toluene: ethyl acetate: formic acid (5:4:1 v/v/v) mixture as the mobile phase were used for the HPTLC method for validation. The plates were scanned at , and the Rf values were 0.690.02 (Diosgenin) and 0.570.02 (Quercetin).
20	A. Srinivas Rao *et al*.,	Simultaneous estimation of quercetin and rutin in ethanolic extract of *Melia azedarach*. Linn leaves by HPTLC method	Precoated silica 60F254 is used as the stationary phase in the HPTLC method for the analysis, and a combination of toluene, ethyl acetate, and methanol (5:3:2) is used as the mobile phase. Quercetin and rutin have Rf values of 0.65 and 0.15 at 254 nm, respectively.
21	Gundu Sindhu Chakraborty *et al*.,	Determination of Quercetin by HPTLC in *"Calendula Officinalis*" Extract	Silica gel 60GF is used as the stationary phase in the HPTLC process, with precoated aluminium plates serving as the mobile phase solvent system. At a wavelength of 366 nm, scanning was done to estimate the Rf value, which was 0.43.
22	Md. Sarfaraj Hussain *et al.,*	Validation of the method for the simultaneous estimation of bioactive marker gallic acid and quercetin in *Abutilon indicum* by HPTLC	The analysis was carried out using silica gel 60 F 254 HPTLC plates with an aluminium foil backing and a solvent system comprising toluene, ethyl acetate, and formic acid (5:4:1 v/v) as the mobile phase. At 270 nm absorbance, the Rf value was 0.31 for gallic acid and 0.50 for quercetin.
23	Jinan Hussain *et al.,*	Qualitative and quantitative comparison of rutin, quercetin and gallic acid concentrations in *Syrian Capparis spinosa*. L Leaves	Silica gel 60 F 254 was used for the analysis, and the mobile phase was a combination of ethyl acetate, glacial acetic acid, formic acid, and distilled water (100:11:11:25). The Rf values at the 366 nm scanning wavelength were 0.39 for quercetin, 0.79 for rutin, and 0.81 for gallic acid.
24	Abhay Prakash Mishra *et al.,*	A Developed and Validated High-Performance Thin-Layer Chromatographic Method for the Quantitative Determination of Quercetin in *Satyrium nepalense* Tubers	Using silica gel 60 F 254 as the stationary phase and the solvent solution toluene: ethyl acetate: formic acid (7:5:1 v/v), HPTLC analysis was performed at 366 nm.
25	Shiv K Yadav *et al*.,	Development and validation of a HPTLC method for simultaneous estimation of quercetin, Chlorogenic acid and trigonelline in polyherbal antibacterial formulation	Using the solvent system chloroform: ethyl acetate: methanol: formic acid (5:3:1.5:0.5v/v/v/v) as the mobile phase, perform HPTLC analysis on aluminium plates 60 F 254. With respect to trigonelline, chlorogenic acid, and quercetin, the well-separated spots were found with Rf values of 0.13, 0.24, and 0.62, respectively.
26	Hiteksha Panchal *et al*.,	Development of Validated High-performance Thin-layer Chromatography Method for Simultaneous Determination of Quercetin and Kaempferol in *Thespesia populnea*	Analysis carried out Using toluene, ethyl acetate, and formic acid (6:4:0.3 v/v/v), silica gel 60 F 254 was previously pre coated on aluminium plates. Quercetin's Rf value at the scanning absorbance wavelength of 366 nm is 0.39, whereas kaempferol's Rf value is 0.50.
27	Khan Dureshahwar *et*	Quantification of Quercetin Obtained from *Allium cepa* Lam. Leaves and its	The quantification was performed using precoated GF254 silica gel plates, a mobile phase solution of

	al.,	Effects on Streptozotocin-induced Diabetic Neuropathy	5:4:1 toluene, ethyl acetate, and formic acid, and a scanning absorbance of 254 nm.
28	Supriya S. Jirge *et al.,*	Simultaneous Estimation of Kaempferol, Rutin, and Quercetin in Various Plant Products and Different Dosage Forms of Bhumi Amla and Amla	The analysis was carried out on precoated silica gel aluminium plates 60 F 254 using a mobile phase containing a mixture of chloroform, methanol, and formic acid (8.2:1.5:1), and the Rf values of kaempferol, quercetin, and rutin at 254 nm are reported.
29	Shikar Verma *et al.,*	HPTLC Analysis for the Simultaneous Quantification of Gallic Acid, Vanillic Acid, Protocatechuic Acid, and Quercetin in the Methanolic Fraction of *Limonia acidissima* L. Fruits	They have used silica gel 60F254 plates for the HPTLC technique. The detection was performed at 254 nm using the solvent mixture of toluene, ethyl acetate, and formic acid (5:4:1), and the observed Rf values are 0.30.00 for gallic acid, 0.470.00 for vanillic acid, 0.370.00 for protocatechuic acid, and 0.420.00 for quercetin.
30	Snehal B. Bhandare *et al.*,	Simultaneous Quantification of Kaempferol and Quercetin in Medicinal Plants using HPTLC.	HPTLC method uses RP-18 F254 plates and toluene, acetone, and formic acid in the ratio 7:3:0.25 as the mobile phase. At the detection wavelength of 254nm, the Rf values for kaempferol and quercetin were determined to be 0.46 and 0.39, respectively.
31	Shikar Verma *et al.,*	Gas Chromatographic–Mass Spectrometric Profile of Non-Polar Fraction and High-Performance Thin-Layer Chromatographic Analysis of Methanolic Fraction with Simultaneous Quantifications of Proto catechuic Acid and Quercetin in *Carissa carandas* L. Fruits	HPTLC analysis employs silica gel 60F254 precoated plates and a solvent solution of toluene, ethyl acetate, and formic acid (6:3:1, v/v). At the highest absorbance wavelength of 310 nm, the Rf values for protocatechuic acid and quercetin, respectively, were 0.57 and 0.61.
32	Nadeem A. Siddique *et al.,*	Simultaneous Quantification of Umbelliferone and Quercetin in Polyherbal Formulations of *Aegle Marmelos* by HPTLC.	Silica gel 60 F-254 was used as the stationary phase for the analysis, which was run on pre-coated aluminium plates. Toluene, ethyl acetate, and formic acid (6:4:1 v/v/v) serve as the mobile phase in the solvent system. The Rf values for umbelliferone and quercetin at 300 nm were reported to be 0.66 and 0.68, respectively.
33	Pushpendra kumar *et al.,*	Simultaneous Quantification of Quercetin and Syringic Acid in Methanolic Extract of *Leucas lavandulifolia* by using Validated HPTLCDensitometric Method	The analysis was performed using toluene, ethyl acetate, and formic acid (7:2.5:0.5 v/v) as the mobile phase on a Silica gel 60 F 254 plate. At the detection wavelength of 275 to 370 nm, the Rf values for quercetin and syringic acid were determined to be 0.32 and 0.41, respectively.
34	Thakshila Aafrin Am *et al.*,	Determination of Quercetin by High Performance Thin Layer Chromatography Method in *Achyranthes Aspera* (L.) Plant Extract.	The test was conducted using a stationary phase of silica gel 60 F 254 and a 5:4:1 combination of toluene, ethyl acetate, and formic acid. At 254 nm, the Rf value 0.60 is found.
35	Avijeet Jain *et al.,*	Simultaneous estimation of quercetin and rutin in *Tephrosia purpurea* Pers by high performance thin layer chromatography.	Precoated silica gel RP-18 F254 was used as the stationary phase for the estimate, and the solvent mixture of methanol, water, and formic acid (40:57:3 v/v/v) was utilized as the mobile phase. The Rf values at 254 nm were 0.07 for quercetin and 0.17 for rutin.
36	Girme AS *et al.,*	Method development, optimization and validation of RP-UFLC method for bioactive flavonoids from *Cassia auriculata*	The analysis was done on an Acquity C18 column using a 0.1% formic acid in water and methanol stepwise gradient elution at a flow rate of 0.350 ml/min. For quercetin QUE-3-O-rutinoside, the highest absorbance at 350 nm wavelength was discovered. For quercetin-3-O-rutinoside and quercetin, the retention times were found to be 3.95 and 5.37, respectively.
37	Shanmugam R *et al.*,	Development and Validation of a RP-UFLC Method for Simultaneous Estimation of Quercetin and Rutin	The reverse phase System C8 column underwent investigation. The mixture of potassium dihydrogen ortho phosphate and acetonitrile in the mobile phase has a 70:30 ratio. For quercetin and rutin, the retention times were 7.4 and 2.8 minutes, respectively, and the detection was carried out at 230 nm.

38	Khaled Elgendy *et al.,*	Analysis of Total Flavonoids in Herbal Drugs Expressed as Quercetin by Reversed Phase-HPLC Method	The analysis was carried out using a Phenomenex column with 0.5% phosphoric acid and methanol as the mobile phase with a flow rate of 1.3ml/min. For quercetin, the method's linearity is established over the concentration range of 240-960 mcg/ml and the retention time was 7.8 minutes.
39	Maric santos *et al.*,	UPLC-MS for Identification of Quercetin Derivatives in Cuphea glutinosa Cham. And Schltdl (Lythraceae) and Evaluation of Antifungal Potential.	The quick C18 analytical column shim pack XR-ODS column underwent analysis. A mixture of acetonitrile: methanol (4:1 v/v) as solvent A and water with 0.1% formic acid as solvent B made up the mobile phase. Full scan mode was used to record the mass spectra in the m/z 200–800 range.

UV Spectrums of Quercetin

The UV-Spectrum of Quercetin, linearity overlay spectrum and Area under curve spectrum of Quercetin is presented in Fig. 31.2, Fig. 31.3 and Fig. 31.4 respectively.

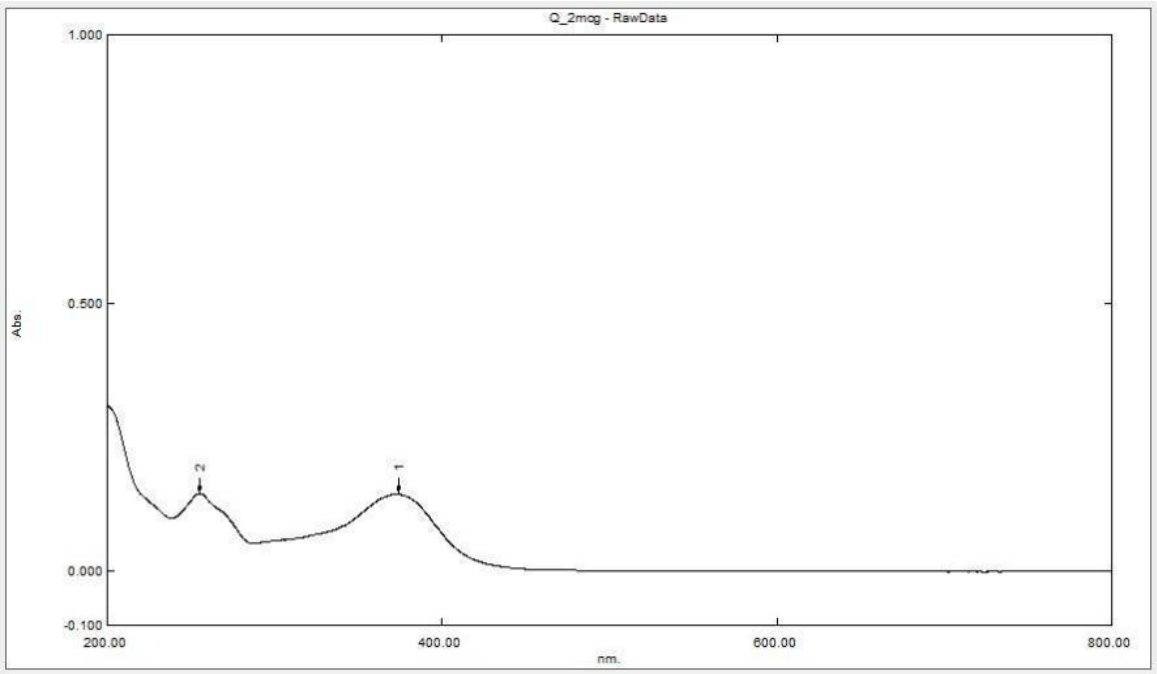


Fig. 31.2 UV-Spectrum of Quercetin

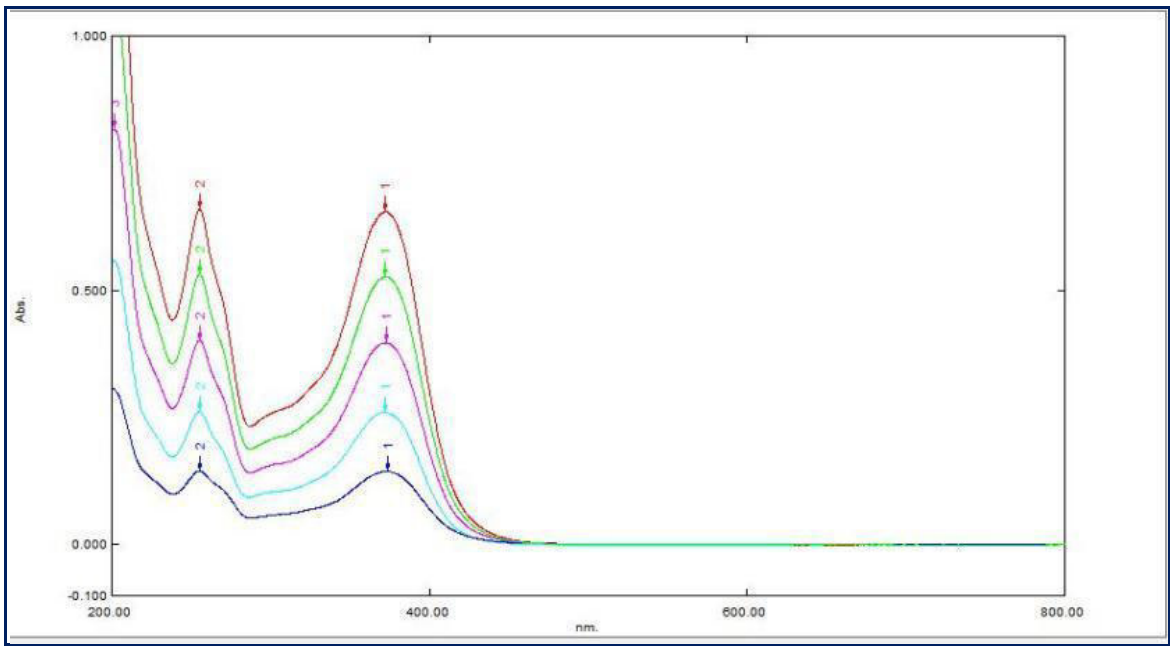


Fig. 31.3 Linearity overlay spectrum of Quercetin

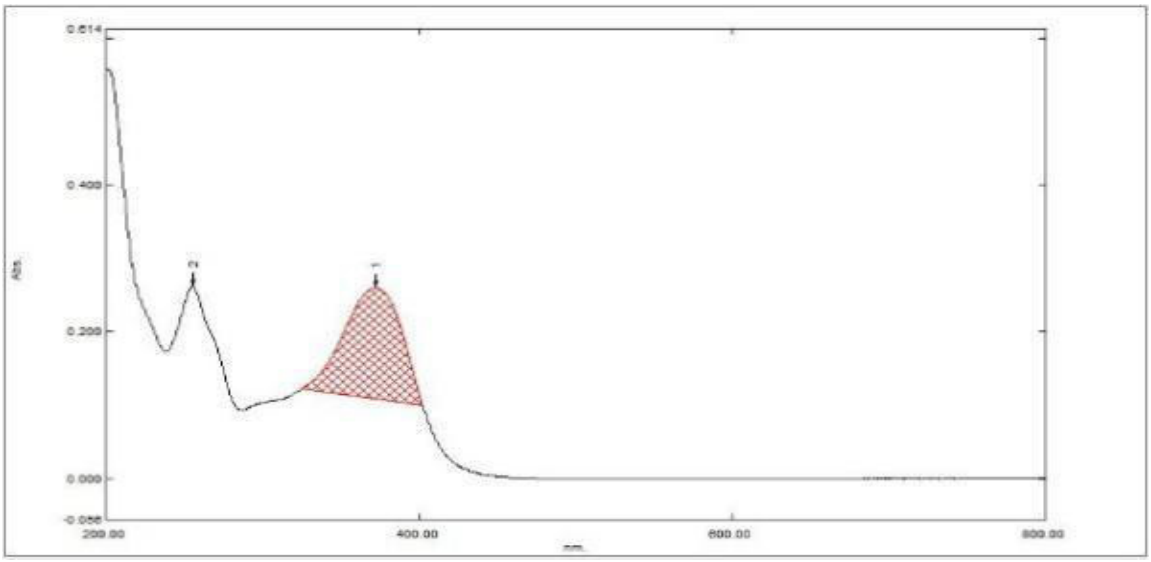


Fig. 31.4 Area under Curve Spectrum of Quercetin

CONCLUSION

For thousands of years, herbal medicine and its preparations have been widely employed in developing and developed countries in the basic health care of society and community. They are in high demand since they are safe and effective, with little side effects or negative repercussions. Ayurveda, Siddha, and Unani are the three major Indian medical systems. Traditional herbal treatments are frequently spontaneously arising and generated from plants, requiring little or no industrial technology in their final manufacturing, and are used to cure illness, diseases, or disorders in local or regional healing practices. Quercetin is a flavonoid generated from plants that has been utilized as a modern pharmaceutical for a range of medicinal uses. The proposed review article discusses Quercetin in detail under various headings such as discovery, pharmacokinetic properties, and physicochemical properties, mechanism of action, synthesis, medicinal values, adverse effects, contraindications, interactions, patents, marketed and novel formulations, patents, future implications, and computational data about drug likeness, pharmacokinetic prediction, molecular docking, and analytical studies on Quercetin. The proposed review article discusses Quercetin in detail under various headings such as discovery, pharmacokinetic properties, and physicochemical properties, mechanism of action, synthesis, medicinal values, adverse effects, contraindications, interactions, patents, marketed and novel formulations, patents, future implications, and computational data about drug likeness, pharmacokinetic prediction, molecular docking, and analytical studies on Quercetin. The proposed review work finds that Quercetin is a well-known plant-derived medication with many therapeutic activities, and so this chemical can be explored in the future in the field of drug design and development.

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Plant metabolites and vegetables for kidney disease prevention and treatment (Book Chapter)

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Abstract

The kidney and the entire excretion system is very much essential in keeping the human body in balance. Many of the major functions of the body are directly or indirectly related to the kidney functions. The disturbance in the kidney functions leads to an imbalance in many other functions. It is very much essential that the kidney function properly. Kidney disease can be prevented and treated by proper management of the diet to a large extent. The intake of carbohydrates, proteins, vitamins, fats, and micro-nutrients (sodium, potassium, and phosphorus) must be monitored properly as these play roles in the functioning of the kidney. The disturbance in the amount of intake might lead to several complications like proteinuria, hyperkalemia, and also fasten the progression of the kidney diseases. Plant-based diets are preferred over animal-based diets in patients suffering from chronic kidney diseases. The individualization of the diet therapy is also recommended to the patients suffering from kidney diseases. © 2025 Apple Academic Press, Inc. All rights reserved.

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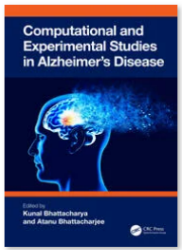

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Computational Modelling of BACE-1, AChE, and Phosphodiesterase Inhibitors as Anti-Alzheimer's Agents

By Vishal S. Patil, Jagadeesh Dodakallanavar, Pukar Khanal, B M Patil, Raushan Kumar Chaudhary, Amit Kumar Shrivastava, Nongmaithem Randhoni Chanu, Darasaguppe R. Harish, Uday Venkat Mateti

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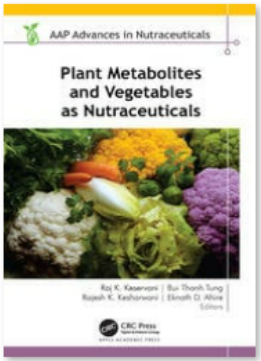

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Plant Metabolites and Vegetables for Diabetes Prevention and Treatment

By Amruta Balekundri, Eknath D. Ahire, Raj K. Keservani

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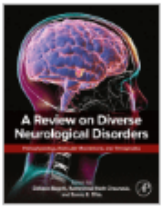

Chapter 20 - Progressive supranuclear palsy

Amruta Balekundri [1], Eknath D. Ahire [2], Khemchand R. Surana [3], Raj K. Keservani [4]

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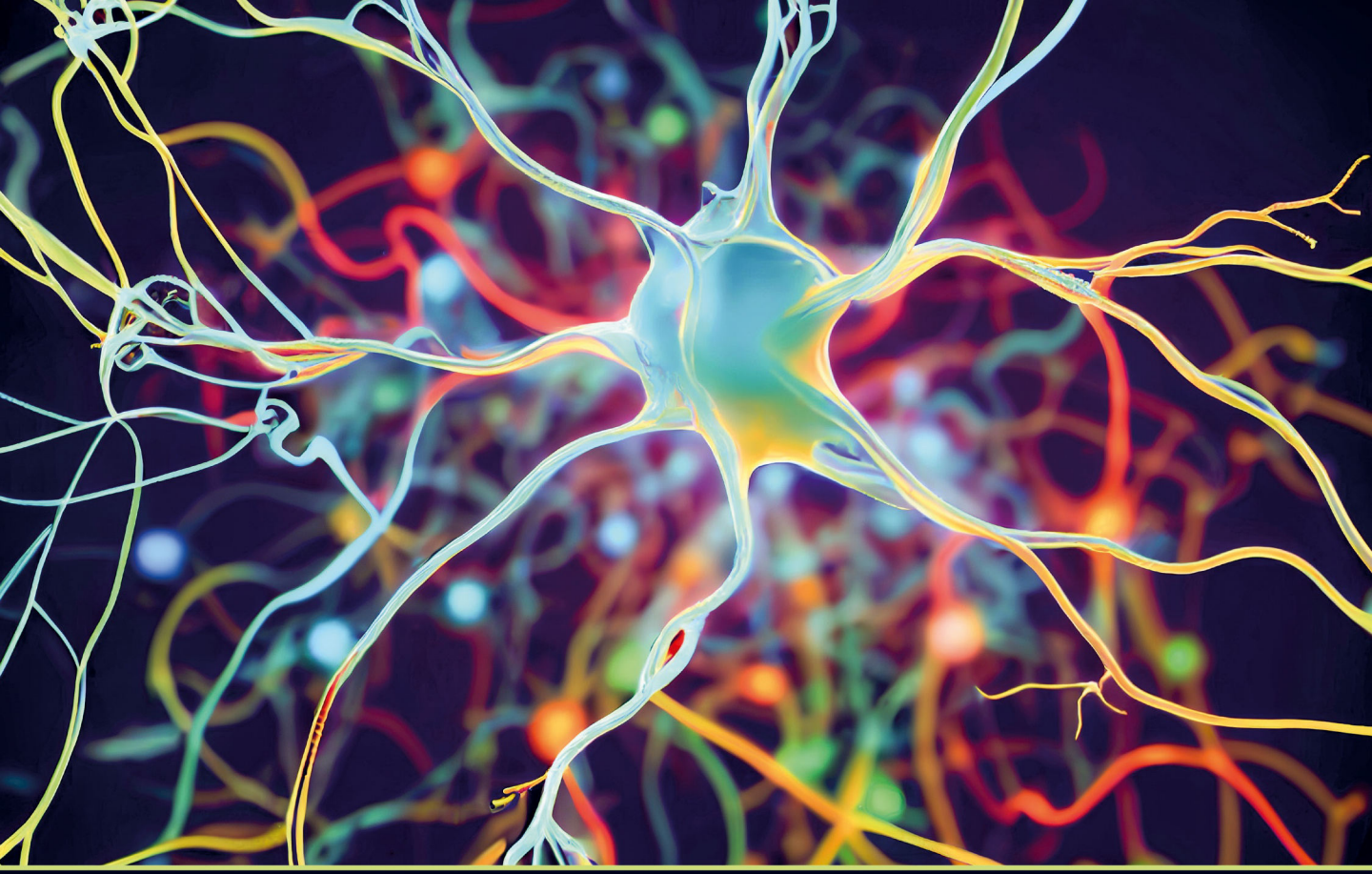
Abstract

Progressive supranuclear palsy (PSP) is the rare condition that occurs when the excessive tau protein gets deposited in the nerve cells, which interferes with the normal functioning of the brain leading to PSP. The exact cause remains unknown, but the factors that are considered responsible for the PSP are genetics, environmental factors, stress, etc. Depending on the pathologies, the PSP are subdivided into various clinical phen Start s. The symptoms associated with PSP are stiffness in muscles, bradykinesia,



THE NEURODEGENERATION REVOLUTION

Emerging Therapies and Sustainable Solutions

EDITED BY
TRIDEVA SASTRI KODURU
RIYAZ ALI M. OSMANI
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CHAPTER

4

Diagnosis and management of neurodegenerative diseases

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4.1 Introduction

Neurodegenerative diseases are characterized by progressive dysfunction and loss of neurons caused by the misfolding and aggregation of proteins. This pathological process leads to a spatiotemporal correlation between the affected central nervous system (CNS) regions and clinical features as a result of this pathological process [1]. These disorders with a peak incidence in later life include a range of neurodegenerative conditions, such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), Pick's disease, dementia with Lewy bodies, and amyotrophic lateral sclerosis. The impact of neurodegenerative disorders is devastating. Patients experience a decline in cognitive abilities, from memory loss to profound dementia, as brain function deteriorates. People with severe motor impairments may have trouble moving around and carrying out daily tasks. Communication and social interaction are frequently hampered, which causes emotional distress and feelings of loneliness. These conditions do not currently have a cure. The treatment goals are to maximize patients' quality of life, minimize disease progression, and control symptoms. A mix of prescription drugs, occupational and physical therapy, and psychological support may be used for this [2].

The prevalence of neurodegenerative diseases is rising worldwide. The prevalence people worldwide currently have dementia, a significant symptom of many neurodegenerative diseases. By 2050, that number is expected to soar to 130 million. The most frequent offender, AD, gradually robs people of their memories and cognitive abilities. Episodic memory loss is the first symptom, followed by language, reasoning, and spatial awareness issues. The second most common, PD, impairs movement and is characterized by stiffness, tremors, and slowness. Although some symptoms can be managed with medication, especially in the early stages of PD, neither disease has a known cure [3].

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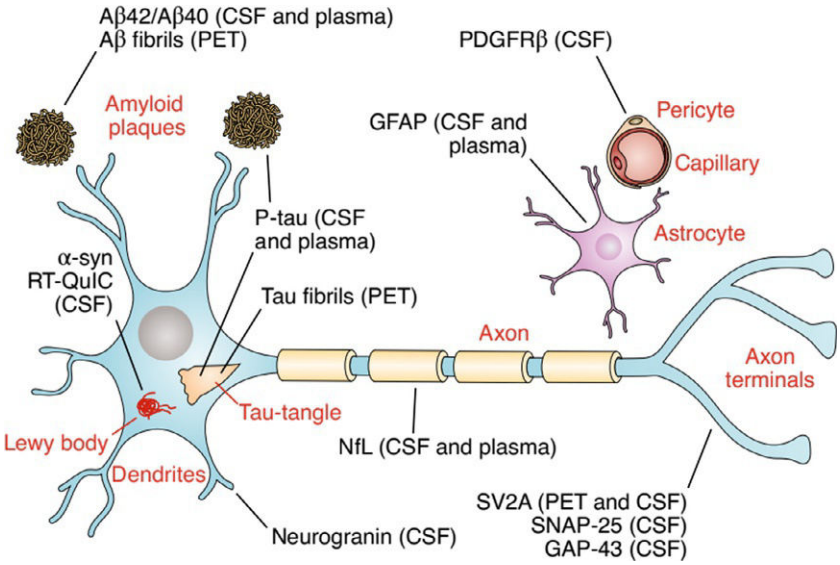

FIG. 1 Biomarkers involved in neurodegenerative diseases [3].

To possibly halt the catastrophic progression of neurodegenerative diseases, early diagnosis is essential. There is a constant search for noninvasive techniques that can be used for early detection when therapeutic intervention shows promise, even though traditional gold standards like neuropathology depend on postmortem analysis. Biomarkers are a very effective technique in molecular diagnostics. Biological molecules that indicate the presence or onset of disease are known as biomarkers. The best candidates have excellent precision and dependability and provide insights into normal and abnormal biological processes. Several methods can measure biomarkers, including imaging techniques like positron emission tomography (PET), magnetic resonance imaging (MRI), and magnetic resonance spectroscopy (MRS). Among these, the marriage of biomarkers with MRS holds particular promise [4] (Fig. 1).

4.2 Alzheimer's disease

4.2.1 Overview of Alzheimer's disease

Alzheimer's disease is the most prevalent form of dementia, which is characterized by memory loss, a steady decline in cognitive function, and, finally, death. From a neuropathological perspective, AD is characterized by specific changes in cortical cell clusters and diffuse brain atrophy. The brain's build-up of abnormal protein aggregates, mainly neurofibrillary tangles and amyloid plaques, is the defining characteristic of AD. These pathological hallmarks lead to the degeneration and loss of neurons in brain regions critical for memory, learning, and thinking.

Early signs of AD can be subtle and include difficulties with language, forgetfulness, and concentration. An individual's capacity to carry out daily tasks and maintain independent

living is impacted by a more noticeable cognitive decline as the disease worsens. Later stages of the illness may cause substantial impairments in thinking, behavior control, and communication [5].

4.2.2 Epidemiology

***Early-onset AD* (*EOAD*):** With an average onset age of 46 years, this less common form of AD manifests before the age of 65. Even though it is uncommon, dominantly inherited familial AD (FAD) brought on by mutations in particular genes (APP, PSEN1, and PSEN2) can show symptoms as early as age 20.

***Late-onset AD* (*LOAD*):** The majority of AD cases (over 95%) are of the sporadic LOAD form, which usually manifests after the age of 65. LOAD is impacted by genetic risk factors, especially changes in the apolipoprotein E gene, even though it is not inherited directly [5].

4.2.3 Diagnosis

4.2.3.1 Clinical evaluation

A comprehensive clinical evaluation for suspected AD involves a multipronged approach encompassing the following critical criteria:

1. Detailed history: Information gathered from a reliable source (family member, caregiver) to understand the patient's disease: general medical history, neurological history, neuropsychiatric history (presence of behavioral or mood changes), family history of dementia, or other neurological disorders.
2. Physical and neurological examination: A thorough physical and neurological exam to assess overall health and identify any neurological signs suggestive of AD.
3. Laboratory tests: Routine tests—complete blood count, metabolic panel (SMA-21), thyroid function tests, vitamin B_{12} and folate levels, and syphilis screening (RPR); Optional tests—erythrocyte sedimentation rate, HIV testing, Lyme disease serology, urinalysis, urine, and drug screen; lumbar puncture (spinal tap)—rarely used; and electroencephalography (EEG)—may help exclude other conditions [7].

4.2.3.2 Neuropathological diagnosis

Alzheimer's disease is characterized by distinct macroscopic features that affect the visual examination of the brain. These features are not specific to AD but provide significant diagnostic clues, mainly when considered alongside clinical symptoms and the absence of other neurodegenerative pathologies.

1. Cortical atrophy: AD brains exhibit widespread cortical atrophy, with a preference for multimodal association cortices and limbic lobe structures, compromising the integration of sensory information, emotional processing, and memory.
2. Regionalized gyral atrophy and sulcal enlargement: Important regions for higher order cognitive activities, the frontal and temporal lobes, exhibit pronounced sulcal enlargement and shrinking of gyri (ridges).
3. Ventricular enlargement: The lateral ventricles, particularly their frontal and temporal horns, grow as the volume of brain tissue declines, reflecting general atrophy.

4. Locus coeruleus (LC) Depigmentation: The loss of neuromelanin pigmentation in the LC, a brainstem region involved in mood regulation and alertness, may be a factor in the cognitive deterioration seen in AD [6].

4.2.3.3 Cognitive assessment

There are several options: the Mini-Mental State Examination (MMSE), the most popular cognitive screening test doctors use for a general cognitive assessment, and the more recent Montreal Cognitive Assessment (MoCA) [8]. The MMSE is commonly used to assess cognitive function and is known to have good test-retest reliability and acceptable sensitivity and specificity. In terms of multidimensionality, two factors were found. The first involved copying a drawing, delayed recall, orientation to time and space, and subtraction with 65% variance. This factor is a reliable marker of cognitive decline across the entire MMSE score range 29 to 10. The second involved denomination, following instructions, naming things, writing and repeating sentences, following a three-step order, and orienting oneself in space with 20% variance. This factor is not a suitable marker of cognitive decline in mild-to-moderate AD. Research suggests that the individual MMSE subtests may have varying degrees of sensitivity in detecting AD [9]. The ceiling effect of the MMSE is one of its drawbacks. The MoCA, on the other hand, was designed as a more challenging test that incorporates executive function, complex visuospatial processing, and higher level language to detect mild impairment with less ceiling effect. The MoCA in mild cognitive impairment (MCI) and AD dementia has been shown to have greater sensitivity in detecting mild levels of cognitive impairment [8].

4.2.3.4 Neuroimaging

Traditional neuroimaging methods include cranial X-ray examination, computed tomography, and routine MRI. When examining the skull with an X-ray, abnormal alterations in the head's bone tissue can be diagnosed with great benefit. MRI can easily separate mild hippocampal atrophy from medial temporal lobe atrophy. Imaging methods used in nuclear medicine include PET and single-photon emission computed tomography (SPECT). Both make use of radionuclide imaging to examine brain metabolism and function, which is very helpful for clinical AD research and early AD diagnosis. MRS is an imaging technique utilizing magnetic resonance and chemical shift phenomena for high-resolution, nondamaging, multidirectional, multiparameter imaging, and brain structure visualization without contrast agents [10,11].

4.2.3.5 Alzheimer's disease neuroimaging initiative

Under the direction of Principal Investigator Michael W. Weiner, MD, the Alzheimer's disease neuroimaging initiative (ADNI) began operations in 2003 as a public-private partnership. First, ADNI participants undergo a series of longitudinal procedures, including blood collection and plasma banking, clinical assessment, neuropsychological assessment, genetics, lumbar puncture for cerebrospinal fluid (CSF; amyloid beta (Aβ) and tau), MRI, PET, AβPET, tau-PET, and at-home digital cognitive testing [11,12].

4.2.3.6 Biomarkers

Diagnosing AD usually happens later on when symptoms start to show. Researchers seek trustworthy biomarkers to detect the disease in its early stages, even before symptoms arise.

Invasive biomarkers

- Locus coeruleus: This area of the brainstem involves memory and attention. Studies suggest that Tau protein deposits (NFTs) accumulate in the LC before appearing in other brain areas during the asymptomatic AD stage. Moreover, LC volume decreases when AD worsens, which may affect cognitive performance.
- Medial temporal lobe and hippocampus: The buildup of NFTs and neurite loss are known to have an early impact on these regions. Hippocampal atrophy has been observed in preclinical AD patients by high-resolution MRI scans, even before the onset of dementia. The degree of atrophy in the hippocampus can indicate whether MCI or AD will develop.
- CSF biomarkers: Although collecting CSF involves an invasive process, it provides essential information on the brain's biochemical alterations that occur in the preclinical phases of AD. Decreased Aβ-42 levels and increased levels of both Tau and phosphorylated Tau protein are recognized CSF indicators.

Noninvasive biomarkers

- *Blood biomarkers*
  1. Aβ proteins: Changes in blood levels of Aβ proteins (Aβ-42, Aβ-40, and Aβ-0) could be a sign of AD pathology.
  2. Tau proteins: Although specificity needs further investigation, elevated blood Tau levels may be a possible indication for AD.
  3. Neurofilament proteins: Higher amounts of neurofilament proteins have been linked to AD, but more research is needed to determine whether AD-specificity applies to these proteins.
  4. Extracellular RNA (exRNA): Brain-derived exRNA in blood may help diagnose AD by providing a window into the expression of genes in the CNS.
- *Saliva biomarkers*:
  1. Phospho-Tau/total-Tau ratio: This ratio appears elevated in AD patients compared to controls, suggesting promise as a biomarker. Further investigation into Tau phosphorylation sites is recommended.
  2. Lactoferrin: Low levels in saliva might be a risk factor for AD.
  3. Acetylcholinesterase (AChE): This enzyme that is involved in acetylcholine breakdown is another proposed candidate.
- *Ocular biomarkers*
  1. Alzheimer's disease affects the eyes in addition to the brain. The brain and retina (eye) both have deposits of Aβ, one of the hallmarks of AD. Patients with AD may develop loss of retinal ganglion cells, optic nerve atrophy, and retinal nerve cell layer thinning, which could affect circadian cycles and vision.
- *Urine biomarkers*:
  1. Oxidative stress Markers: Patients with AD may have higher levels of molecules such as isoprostanes in their urine, which show oxidative damage and may signal the advancement of illness.
  2. AD7c-NTP protein: This protein, which is increased in the urine of AD patients, may be associated with the amyloid plaques characteristic of AD [13].

4.2.4 Management

4.2.4.1 *Cholinesterase inhibitor*

These medications are currently thought to be the standard of care for AD. The US Food and Drug Administration (FDA) has approved ChEIs for treating mild-to-moderate AD. They are galantamine, rivastigmine, donepezil, and tacrine. These substances prevent acetylcholine from being broken down, which raises its concentration and lengthens its activity at synapses [14,15] (Table 1).

4.2.4.2 *Herbal drugs for the management of AD*

There are common traditional Indian plants that can be utilized to treat neurological illnesses, including dementia and Alzheimer's. Ayurvedic rasayana medications are abundant in immunomodulatory and antioxidant compounds. Their primary mode of action might be their capacity to scavenge free radicals or activate the oxidant defenses of cells [16] (Table 2).

4.2.4.3 *Nonpharmacological management*

Sleep disturbances are linked to AD pathophysiology, with improper sleep accumulating Aβ, potentially triggering memory decline and converting to AD. Nonrapid-eye-movement sleep disruptions are a novel factor linking cortical Aβ to impaired hippocampus-dependent memory consolidation. Proper sleep in older age is a new treatment target, offering preventative and therapeutic benefits.

Physical activity (PA) has been linked to brain health, particularly in reducing the risk of neurodegenerative dementia-causing AD. Moderate PA, including aerobic exercises, resistance training, stretching, and toning, can improve physical and mental health in older adults.

TABLE 1 Cholinesterase inhibitors for AD management.

Drug	Dose	Side effects
Tacrine	Starting dose 10 mg 4 times daily and increased dose to 40 mg/day to a maximum daily dose 40 mg 4 times daily	Hepatotoxicity
Donepezil	The starting dose is 5 mg/day, and bedtime is usually with or without food. Increased up to 10 mg/day.	Nausea, vomiting, diarrhea, anorexia, insomnia, muscle cramps, fatigue
Rivastigmine	The starting dose is 1.5 mg twice a day with meals (breakfast and supper), and this dose is increased by 3 mg/day, not faster than every 4 weeks, to a therapeutic dose of 6–12 mg/day.	Nausea, vomiting, anorexia, diarrhea, fatigue
Galantamine	The starting dose is 4 mg twice daily, which is increased every 4 weeks. The therapeutic dose is 16–24 mg/day.	Nausea, vomiting, anorexia, weight loss, syncope, fatigue, dizziness, dyspepsia, diarrhea
Memantine	The recommended starting dose for memantine is 5 mg/day (once daily), with or without food. The recommended target dose is 20 mg/day.	Headache and confusion may be observed during the titration phase and is usually transient

TABLE 2 Herbal medicines are used for AD management.

Herb name	Activity
Ashwagandha (*Withania somnifera*)	Ashwagandha may help restore damaged neuronal circuits by extending its neurite outgrowth.
Brahmi (*Bacopa monniera*)	This medicinal herb acts as a mental tonic and a nervine and can be used for the treatment of cognitive and neurological disorders
Gotu kola (*Centella asiatica*)	It has been used to restore youth and memory and to strengthen the nervous system. It has been used to boost memory.
Chandan (*Santalum album*)	The potential of this plant is to increase memory and cognitive function. It significantly improves learning and memory in scopolamine-induced dementia.
Bhalawa (*Semecarpus anacardium*)	It acts as a brain tonic and is a potent antioxidant agent.
Haldi (*Curcuma longa*)	Curcumin, when administered in a mouse model of AD, decreased serum Aβ levels as well as minimized the burden of Aβ in the brain, and this effect is seen mainly in the neocortex and hippocampus of the AD mouse model

Although the exact mechanisms behind this decrease in dementia risk are not fully understood, it could be a crucial behavioral factor in healthy aging [16].

Cognitive stimulation is a nonspecific approach focusing on social interaction originating from Reality Orientation Therapy and Reminiscence Therapy. It involves daily group sessions over 2 months, stimulating global functions, ideomotor praxis, visual, auditory, spatial memory, selective attention, verbal language, and constructional praxis. Neuropsychological assessments are performed at baseline and after the program's completion. Cognitive rehabilitation is a person-centered approach for impaired abilities, aiming to improve strategies involving memory, task sequencing, and lists. It is more effective for individuals suffering from stroke or traumatic brain injuries than for AD. Ethical considerations are crucial in evaluating the efficacy of these treatments.

Virtual reality (VR) is a three-dimensional (3D) interactive and multisensory environment created by a computer from numerical data. It includes various systems such as immersive VR, Computer-Assisted Virtual Environment (CAVE), augmented reality, and desktop VR. VR has been used in medicine for teaching anatomy, training in diagnostic procedures, and rehabilitation. It allows for tailor-made exercises with meaningful environments, such as auditory-visual aversive stimuli modulating fear. VR has been used in AD to improve cognitive/behavioral and functioning domains. Spatial memory impairment, a deficit in mental frame synchronization between egocentric and allocentric spatial representations, is a significant issue in AD. VR can replace the need for appropriate neuropsychological investigations for spatial memory, providing potential for neurorehabilitation. A VR day-out task environment in patients with incipient AD could provide additional predictive information. Implementation of VR with EEG and neuroimaging is also considered. Custom-designed 3D video game training in aging has shown performance benefits, improved cognitive abilities, and increased electroencephalograms [17].

4.3 Parkinson's disease

Parkinson's disease is a degenerative neurological condition marked by stiffness, tremor, and slowness of movement. It is linked to loss of neurons in the substantia nigra and other brain regions. Nonmotor characteristics, including dysautonomia and dementia, are common, particularly in the later stages of the illness [18]. With over 6 million cases worldwide, PD is the second most prevalent neurodegenerative illness. Men are more likely than women to get PD, with a prevalence ratio of roughly 3:2. Age is the primary risk factor for the disease. The motor disturbances lead to a declining quality of life and a growing disability that makes it difficult to carry out daily tasks. The development of postural instability, growing gait difficulties, dysphagia, and dysarthria, along with the early occurrence of classic motor symptoms, form the foundation of the diagnostic criteria and drive the advancement of motor disability [19].

4.3.1 Diagnosis

4.3.1.1 Genetic testing

Parkinson's disease is a sporadic neurodegenerative disorder characterized by the loss of midbrain dopamine neurons and Lewy body inclusions. The disease is thought to result from a complex interaction between multiple predisposing genes and environmental effects. Genetic testing for mutations in seven genes, including—synuclein, UCHL1, NURR1, LRRK2, DJ1, PINK1, and Parkin, is of little clinical relevance. However, confirmation of this recessive form of disease might be helpful in genetic counseling. Recently, the gene encoding a leucine-rich repeat kinase (LRRK2) has been identified in several families with autosomal dominant Parkinsonism, suggesting that screening for LRRK2 mutations could be important in the diagnostic work-up and counseling of patients with PD.

4.3.1.2 Clinical physiology

Neurophysiological tests, such as long latency-reflex times and transcranial magnetic stimulation, can reveal subcorticomotor or corticomotor loop dysfunction abnormalities. Some abnormalities are suggested as diagnostic markers for atypical Parkinsonian disorders, but none are concrete for degenerative Parkinsonism, making clinical neurophysiology in routine clinical practice insufficient.

4.3.1.3 Olfactory test

Ninety per cent of patients experience hyposmia, or the loss of smell, as an early symptom of PD. It frequently manifests before motor symptoms. Hyposmia appears to be PD-specific. In other Parkinsonian conditions, it occurs less frequently and is milder. According to a study, PD eventually struck certain hyposmic people, who had brain abnormalities connected to the condition. Sniffin' Sticks and UPSIT tests effectively detect odor issues in PD. According to these results, smell testing is helpful for early PD screening. More effective illness management is made possible by early identification [18].

4.3.1.4 Neuroimaging

Traditional structural imaging does not generally reveal significant changes in PD. However, recent studies have shown reduced fractional (FA) in the region, which is thought to

correspond to ascending nigrostriatal fibers and improved sensitivity and specificity for PD. Diffusion tensor imaging measures of nigrothalamic connectivity combined with a modified T1 nigral volumetric study have also been suggested. MRI has been used to demonstrate changes in thalamic shape in patients with PD. Resting-state functional MRI has shown reduced connectivity in the supplementary motor area, left dorsolateral prefrontal cortex, and left putamen in PD patients studied off-medication. In contrast, connectivity increased in the left cerebellum, primary motor, and parietal cortex. Functional MRI (fMRI) can study activation patterns during motor or cognitive tasks but is sensitive to the effects of catechol-*O*-methyltransferase (COMT) polymorphisms [20].

Parkinson's disease was diagnosed with the first SPECT tracer, 3-iodo-6-methoxybenzamide (IBZM), which was tagged with iodine-123. When compared to PD, parkinsonian disorders with striatal pathology have lower levels of IBZM, which binds to D2-dopamine receptors in the striatum. Tropane derivatives (-CIT) and FP-CIT (DaTSCAN), two presynaptic dopaminergic terminal SPECT ligands that exhibit asymmetric decreases in striatal DAT binding with SPECT, even in very early stages of illness, have been put into clinical practice. Although DAT-SPECT imaging has been employed as a stand-in marker for advancing illness, the sensitivity problem remains unresolved. Due to sensitivity or clinical diagnostic accuracy issues, approximately 10% of patients with de novo PD, who have received a clinical diagnosis, may exhibit normal DAT binding using existing SPECT tracers.

Parkinson's disease patients have been found to have typical findings on PET, with declines in putamina Ki-values of ^{18}F-dopa uptake linked with the severity and duration of the condition. On the other hand, regular ^{18}F-dopa PET readings may be found in as many as 10% of those who have been clinically diagnosed with PD.

Brain parenchyma can be seen using transcranial B-mode Doppler sonography; research indicates that hyperechogenicity of the substantia nigra is observed in over 90% of patients with a clinical diagnosis of PD. This result is associated with decreased ^{18}F-dopa uptake with PET and poor performance on specific motor tasks. More research is required to determine its usefulness as a screening tool for people at risk of the illness [19].

4.3.2 Management

4.3.2.1 Pharmacological management with drugs

For PD, levodopa is the most popular and successful pharmacologic treatment for symptoms. Parkinson's disease, bradykinesia, stiffness, and tremor are levodopa-responsive symptoms. Oral preparations frequently give mixed levodopa with peripheral DOPA decarboxylase inhibitors, which raises levodopa's availability in the CNS. To lower the risk of adverse medication reactions (such as nausea, hallucinations, and postural hypotension), especially in the elderly, oral levodopa is generally started at a low dose and gradually increased. A standard starting dosage for Sinemet/Madopar would be 62.5 mg (12.5 mg DOPA decarboxylase inhibitor/50 mg levodopa) once a day. After 5–7 days, the dosage would be increased as tolerated in increments of 62.5 mg to an initial maintenance of 3 times daily. Dyskinesia may manifest during the peak concentrations of levodopa in plasma. In cases with advanced levodopa-responsive PD, carbidopa-levodopa gel can be administered continuously via the duodenum (Duodopa) when other medicinal treatments have failed [22] (Table 3).

TABLE 3 Pharmacological management with drugs in PD. [21,23,24]

Class	Drug	Indication	Side effects
Levodopa/ carbidopa:	Levodopa Levodopa gel Inhaled-Levodopa	Initial therapy	Dyskinesia, Nausea
Dopamine agonists	Pramipexole (0.125 mg, 3 times/day, increasing weekly)	Initial/adjunctive	Nausea
	Ropinirole (0.25 mg, 3 times/day, increasing weekly)	Initial/adjunctive	Drowsiness
	Rotigotine 2 mg/24 h	Initial/adjunctive	Hallucination
	Apomorphine	Rescue therapy for off period	Nausea, drowsiness, hallucination
COMT inhibitors	Entacapone (200 mg with levodopa)	Adjunctive	Diarrhea, dyskinesia
	Tolcapone (100 mg, 3 times/day) Opicapone (50 mg every night)	Adjunctive Adjunctive	Dyskinesia, hepatotoxicity, dyskinesia
MAO inhibitors	Rasagiline (1 mg every morning)	Initial/adjunctive	Dry mouth
	Selegiline (5 mg, 2 times/day)	Initial/adjunctive	Mood changes
	Safinamide (50 mg/day)	Adjunctive	Dyskinesia
Others	Amantadine	Adjunctive	Skin rash, hallucination
	Istradefylline (20 mg/day)	Adjunctive	Hallucination
	Clozapine (12.5–25 mg every night)	Adjunctive	Constipation, tachycardia

COMT, *catechol-O-methyltransferase;* MAO, *mono amino oxidase.*

4.3.2.2 Advanced therapies for PD

Deep brain stimulation (DBS) is a promising therapeutic strategy for PD, with patients experiencing fluctuating responses, troublesome dyskinesia, or levodopa-unresponsive symptoms. Ablative surgical approaches like stereotactic destruction of overactive brain nuclei have been replaced by DBS using implanted pulse generators. The main advantage of DBS is that stimulation parameters can be customized to the patient's needs, optimizing benefits. Thalamic DBS is most frequently used to control high-amplitude tremor in patients with essential tremor. At the same time, STN or globus pallidus interna are the most frequent targets for DBS treatment of patients with PD with disabling tremors or levodopa-related motor complications. Some patients have found benefit with unilateral focused ultrasound lesioning of the STN or thalamus (in tremor-dominant forms of PD), especially if their symptoms are noticeably asymmetric. Finally, patients with PD, who are most affected due to their gait issues are increasingly being investigated for spinal cord stimulation [21].

Advanced therapies should be taken into consideration if considerable off-time or dyskinesia continues after optimal oral treatment. The least intrusive and most straightforward to use is apomorphine, a potent dopamine agonist administered by continuous subcutaneous

infusion. Extensive clinical experience was followed by the recent double-blind TOLEDO research, which verified that apomorphine significantly reduced off-time and improved on-time compared to placebo [23].

4.3.2.3 Nonpharmacological management in PD

More people are realizing the value of exercise in managing PD. According to recent NICE guidelines, a PDNS should be available to all individuals with PD. Additionally, referrals for specialized physiotherapy, speech and language therapy, and occupational therapy should be made for individuals with trouble with their daily activities of living, gait/falls, and communication/swallowing, respectively. Although they may aid in the creation of care plans at any stage of PD, palliative care services are becoming increasingly important in the palliative stage of the disease and end-of-life care [23].

4.4 Huntington's disease

The most prevalent hereditary neurodegenerative disease, HD, is characterized by uncontrollably excessive motor activities as well as cognitive and emotional abnormalities [26]. Huntington's disease is autosomal-dominant and has a unique phenotype that includes chorea and dystonia (an involuntary brief movement that tends to flow between body regions), incoordination, cognitive decline, and behavioral difficulties. An extended CAG repeats at the N-terminus of the mutant protein known as huntingtin causes a polyglutamine strand with a variable length. There is evidence to imply that this tail adds a harmful role [25]. In the context of a family history, HD is usually diagnosed based on clinical signs and may be verified by genetic testing. Family members at risk can use predictive testing, but qualified professionals should only do the testing and counseling. Numerous brain regions undergo degeneration, primarily about the neurotransmitter's glutamate, dopamine, and γ aminobutyric acid [27].

CAG repeat length and its clinical significance [28].

≤26—Normal
27–35—Intermediate.
Not pathological, but may cause disease through: ▸ Expansion into pathogenic range in successive generations ▸ Genomic instability resulting in mosaicism with expansion into pathogenic range in some neuronal populations, leading to clinical features of HD in rare cases
36–39 Pathogenic with incomplete penetrance
≥40 Pathogenic with complete penetrance

4.4.1 Diagnosis

HD diagnosis is a multistep procedure. Initially, physicians search for a mix of cognitive decline, mental disorders, and mobility concerns. A strong family history strengthens the case for HD. Given the potential consequences for the patient and family, informed permission is

essential before any testing, particularly genetic testing. Blood tests and brain imaging help rule out other illnesses but are not diagnostic for HD. The most accurate diagnosis can be made through genetic testing. But, since there isn't a treatment now, it 'is a personal decision. Genetic counseling can help people make this choice [29].

4.4.1.1 Clinical diagnosis

The Unified Huntington's Disease Rating Scale (UHDRS) and Total Motor Score (TMS), now the gold standard for diagnosing HD, has limitations when identifying modest motor abnormalities in premanifest gene carriers. This lower sensitivity makes it more challenging to diagnose HD early on, which is essential for treatment interventions and clinical studies. A more sensitive and objective method has been made available with the advent of quantitative motor tests. These assessments use computer-based analysis to accurately quantify mild motor impairments, including fingertip coordination and chorea. Chorea can be recorded objectively and precisely by chromatography. Interestingly, Q-Motor tests have succeeded in sizable observational studies, demonstrating their efficacy in detecting HD before symptoms appear. Researchers can attain more accurate early diagnosis, improve patient care, and create more potent HD therapy methods by combining objective and measurable Q-Motor tests [30].

4.4.1.2 Prenatal diagnosis

A diagnosis made before conception is also feasible because the test can be run on any cell with a DNA-containing nucleus. Chorionic villus sampling can be done between weeks 10 and 12, amniocentesis between weeks 15 and 17, and DNA testing between weeks 16 and 17. The procedure is only started if the parents are already aware to prevent two people from learning their genetic status simultaneously. If the HD gene is discovered in the embryo, terminating the pregnancy starts. The mother cannot accept this decision [29].

4.4.1.3 Differential diagnosis

Patients do not have the causal huntingtin gene expansion (mHTT), but they do have the characteristic triad of HD symptoms: chorea, cognitive impairment, and mental disorders. Since the genetic characteristic is missing, different diagnostic strategies are required to determine the underlying cause. Only 2%–4% of cases result in a good diagnosis, making it difficult to determine the source of a suspected HD phenocopy. Several genetic abnormalities can manifest as HD phenocopies; in Western populations, the most common culprits are spinocerebellar ataxia 17 (SCA17) and C9orf72 expansion. Dentatorubral-pallidoluysian atrophy should be taken into account in the differential diagnosis for patients, who report seizures. HD phenocopies can result from etiologies other than genetics. Investigation is warranted when abnormal MRI results indicate iron deposition illnesses, including neuroferritinopathy and neurodegeneration with brain iron accumulation. Another possible explanation of peripheral blood smear analysis is the presence of acanthocytes, a hallmark finding in neuroacanthocytosis [31].

4.4.2 Management

Many agents and surgical procedures have been evaluated in HD for their antichoreic efficacy, including dopamine-depleting agents, dopamine antagonists, benzodiazepines, glutamate antagonists, acetylcholinesterase inhibitors, dopamine agonists, antiseizure

medications, cannabinoids, lithium, DBS, and fetal cell transplantation. Pharmaceutical therapies are commonly used to treat hyperkinetic movement disorders, such as chorea, dystonia, ballism, myoclonus, and tics. However, they may also have an impact on psychiatric problems, such as mania, apathy, melancholy, anxiety, or obsessive-compulsive disorder, as well as cognitive loss linked to HD. In addition to behavioral regimens, cognitive interventions, alternative and complementary therapies, and adjunctive therapies are essential in treating HD symptoms [32].

- Drug treatment for chorea: Tiapride, Olanzapine, Tetrabenazine, Pimozide, Risperidone, Fluphenazine.
- Drug treatment for depression: citalopram, fluoxetine, Mirtazapine, valproinezur, and carbamazepine.
- Drug treatment for aggression: citalopram, Sertraline, Olanzapine, Dipteron, Haloperidol [29].

Tetrabenazine: Patients with HD are allowed to utilize TBZ, an inhibitor of vesicular monoamine transporter and a dopamine-depleting drug that has been shown in a controlled trial to reduce chorea significantly [29]. The most common side effects are depression and sedation. TBZ verified its effectiveness on chorea, although withdrawals from side effects were standard. In a long-term efficacy retrospective research, 63 patients receiving TBZ medication for an average of 34 months demonstrated a durable impact on chorea, but with a gradual diminishment with time [33].

Antipsychotics: There is no typical antipsychotic drug found effective in placebo-controlled studies. In one of the studies, the haloperidol drug administered at 1.5–10 mg/day responded to a 30% reduction in chorea. The effect of Olanzapine in small open-label studies on motor symptoms associated with HD was 0%–66%. Other drugs like Quetiapine, Clozapine, and Aripiprazole also show significant effects but are associated with multiple adverse effects [32].

4.5 Conclusion

Huntington's, Parkinson's, and ADs are crippling illnesses for which there is currently no treatment. However, there is optimism for better patient outcomes due to breakthroughs in diagnosis and therapy. Early diagnosis enables early symptom management and intervention, which may impede the course of the disease and enhance quality of life. Finding a cure for these illnesses and creating more potent therapies will need ongoing study into their fundamental origins.

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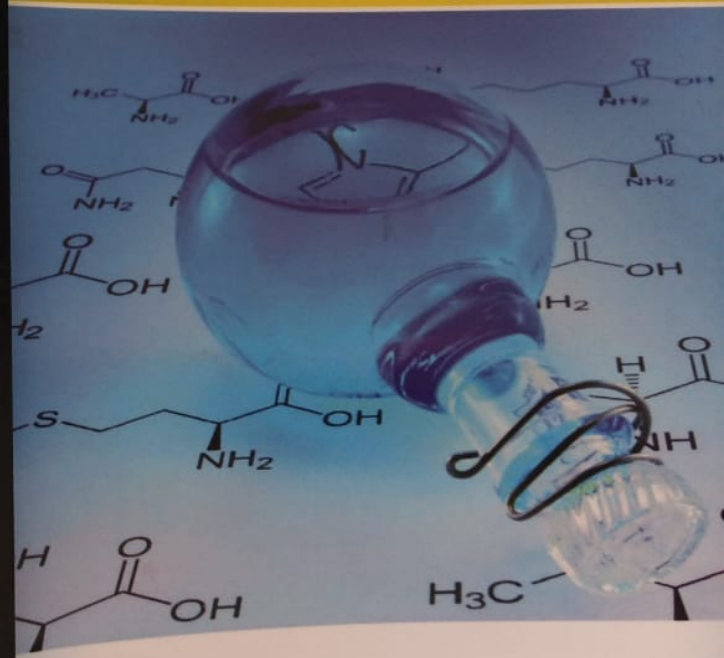

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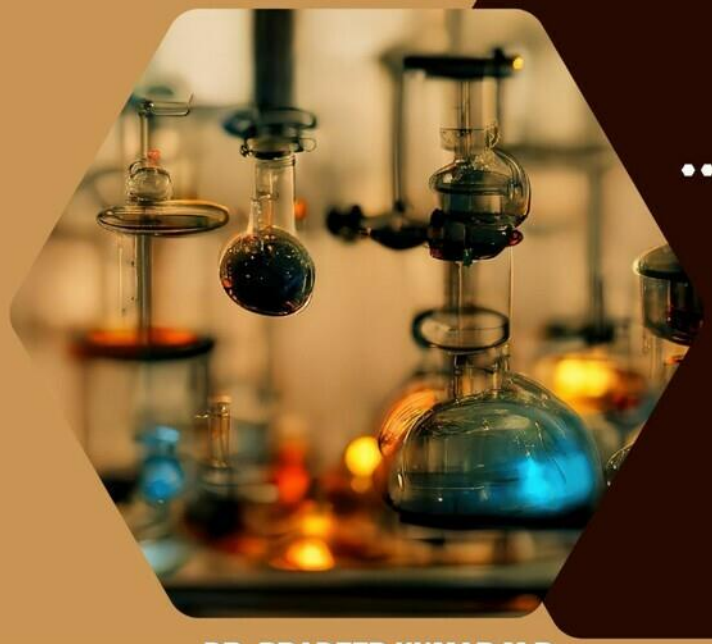

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Chapter 11
Versatile and Emerging Applications of Halloysite Nanotubes: From Nanotechnology to Biomedicine and Environmental Solutions

Revati Dharampal Sagare, Fatima Sanjeri Dasankoppa, Hasanpasha N. Sholapur, M. Mahesh, and N. G. Nanjunda Swamy

Abstract Halloysite nanotubes (HNTs), are 1D naturally occurring aluminosilicate clay minerals mined from the natural clay deposits. These nanotubes possess a distinct hollow nano-tubular morphology, with their length ranging from 400 to 1000 nm and an inner diameter of 10–20 nm as well as an outer diameter of 40–80 nm. HNTs are natural in origin, bio-compatible, bio-degradable and eco-friendly in nature. The unique structure and distinctive characteristics of HNTs contributes to a tunable surface chemistry with an enhanced surface area. The presence of large lumen diameter and nano porous structure of HNTs provides an active site and make HNTs as a potential nano-container to encapsulate a variety of anti-inflammatory, antibiotics, anti-cancer and anti-fungal drug moieties. This book chapter outlines the recent advances and applications of HNTs in the various domains of bio-medicine and non-bio-medicine. Their biocompatibility and ability to encapsulate drugs, enzymes, and other bioactive agents make HNTs valuable in drug delivery systems, where they provide controlled and sustained release profiles. Additionally, the applications of HNTs are explored for environmental applications such as waste water treatment and fabrication of eco-friendly as well as bio-degradable food packaging materials. This chapter delivers an insightful resources to the researchers in the design and development of an innovative solutions for a wide range of applications.

Keywords Halloysite nanotubes · Food packaging · Tissue engineering · Wound healing · Environmental science · Drug delivery

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CHAPTER 13

Data Interpretation and Management Tools for Application in Pharmaceutical Research

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Abstract: The information flow in pharmaceutical research before data interpretation and management was largely manual and simple, with limited application of technology. Establishing the research objective, designing the study, collecting data, analyzing data, and interpreting the result were laborious, tedious, and time-consuming processes. Manually entering and sorting a large amount of data made researchers more prone to human errors, leading to incorrect and invalid results. The chapter draws on data mining, data abstracting, and intelligent data analysis to collectively improve the quality of drug discovery and delivery methods. To develop new drugs and improve existing treatments, software can be used to analyze large datasets and identify patterns that help understand how drugs interact with the body. Virtual models of organs and cells are employed to study the effects of drugs, automate drug testing, and predict adverse drug reactions. Pharmaceutical management tools, such as pharmacy management software, electronic prescription software, inventory management software, and automated dispensing systems, are highly valuable for managing inventory, tracking patient prescriptions, monitoring drug interactions, maintaining patient information and history, and providing up-to-date drug information. The main objective of this chapter is to highlight the various tools and software solutions available and how they can facilitate the research process to ensure compliance with relevant regulations and laws regarding human healthcare safety.

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Keywords: Automated dispensing system, Electronic prescription software, Intelligent data analysis, Inventory management software, Pharmaceutical management tools, Virtual model.

1. INTRODUCTION

Pharmaceutical research is a crucial field of study because it involves the constant development of new medicines and treatments for a wide range of medical conditions. This process demands extensive experimentation and analysis to identify novel potential treatments and assess their safety and efficacy [1].

These tools are intended to boost research productivity and success, producing more trustworthy and accurate results. This has led to an increased reliance on cutting-edge technology and software in pharmaceutical research, allowing scientists to study complex biological systems and create new treatments and pharmaceuticals more swiftly than before. This essay will discuss some of the relevance.

Interpreting data is an essential part of pharmaceutical research and is necessary for drawing valid conclusions about the development, efficacy, and safety of medications. The increasing volume of data generated by contemporary pharmacological research has made data interpretation more challenging [2]. It involves combining data from various sources to make informed judgments that can guide the creation of novel therapies and drugs. Effective data interpretation in the pharmacy sector requires a combination of specialized expertise, scientific acumen, and subject knowledge. Additionally, examining complex data sets and drawing conclusions necessitate the use of sophisticated software and statistical techniques. The importance of accurate data comprehension in this context cannot be overstated since it enables scientists to make decisions regarding patient care, public policy, and the course of action based on readily available facts. The highest level of patient care and the financial sustainability of healthcare organizations depend on the efficient management of pharmaceutical resources, including medications, equipment, and personnel. Pharmacies employ various management technologies, such as staff scheduling software, performance monitoring tools, and inventory management systems to achieve these objectives [3]. These tools assist pharmacists in streamlining their processes, reducing waste, and improving patient outcomes. This article outlines the frequently used management tools in pharmacies and describes their applications in diverse pharmacy contexts. It also examines the advantages and challenges associated with implementing these innovations, highlighting the importance of thorough planning and research when introducing new administrative systems. This study

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underscores the significance of management tools in optimizing pharmaceutical operations and enhancing patient care.

2. HISTORY

Data management and storage require specialized equipment. Various methodologies have been employed throughout the history of medical research, and contemporary technology has also simplified the management of complex data. Edward H. Shortliffe examines the importance of striking a successful balance between modern thinking methods and advancements in information infrastructure, adherence to international standards, and education [4].

2.1. Conventional Approach

In the past, research in medical science relied solely on manual data entry and data screening methods. Since 1993, research data and information processing have undergone significant advancements. In fact, the data collected and exchanged across various research laboratories and healthcare facilities are evolving rapidly. What used to be isolated databases or laboratory information systems are now integrated into departmental, hospital, community, and research-based medical information systems. With the increasing volume of data, extracting relevant information for decision support has become increasingly challenging. Traditional manual data analysis is no longer sufficient, necessitating the development of efficient computer-based analysis techniques. Two notable examples of these techniques are data abstraction and data mining [5].

2.2. Computer and Software Resolutions

Artificial intelligence serves as a reminder that computers in medicine are by no means a recent development. Numerous industries use computers in similar ways, and few technical advances have been explicitly sparked by what can be called “business computing” in the medical field. It doesn't appear realistic that these computerized medical applications will “reshape” medicine, nevertheless. Computers haven't significantly changed the kind of decisions made or how they are made during the previous 20 years.

We recognize that the lack of valid perspectives on enhancing executives' critical thinking abilities can significantly exacerbate this issue [6]. Similarly, during that time, most medical business computers had a minimal impact on the work of doctors. A secondary, less common use of computers in medicine was focused on the content rather than the format of healthcare. Computers were primarily tasked with maintaining medical records, laboratory data, clinical trial information, and other data, provided they could effectively manage billing records. Furthermore, if

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beneficial for data storage, a computer should also assist in data analysis, organization, and retrieval. To date, this second category of medical computing has been primarily addressed through the use of decision theory, comparison of new cases with extensive databases of past cases, and the utilization of flowcharts or clinical algorithms as the three primary approaches.

3. TYPES OF RESEARCH DATA

There are two main types of research data: qualitative data and quantitative data.

3.1. Qualitative Data

Qualitative data are non-numerical and descriptive in nature. This type of data is often gathered through methods such as interviews, focus groups, observations, and other approaches that involve direct interaction with participants [7]. Qualitative data are used to gain a comprehensive understanding of participants' experiences, attitudes, and behaviors. Examples of qualitative data include interview transcripts, observational notes, and field notes.

3.2. Quantitative Data

Quantitative data are numerical and can be measured and statistically analyzed. This type of data is often collected through methods such as surveys, experiments, and other approaches that involve quantifying variables of interest [8]. Quantitative data can be utilized to identify patterns and relationships among variables and to test hypotheses. Examples of quantitative data include survey responses, test scores, and measurements such as percentage and weight.

Another kind of data that can be used in research is mixed-methods data, which is data from both qualitative and quantitative sources. These are the most typical types of research data. Two additional categories of data exist: secondary data, which can be used in research, and meta-data, which is information about data that can be used to understand how research studies were conducted and how data were gathered and analyzed. Information that has been proactively gathered for one more purpose and can be used in research is considered optional information. For a better understanding of research data management, the five major types of research data are as follows:

3.3. Observational Data

This type of data is collected through the observation of people, objects, or phenomena without any form of manipulation or intervention. Various techniques, including direct observation, video recordings, and other methods, can be employed to gather observational data [9].

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3.4. Experimental Data

Experiments are conducted to gather this type of data, involving the manipulation of one or more variables and the observation of how these changes impact other variables. Experimental data are usually collected in laboratory settings, but they can also be conducted outdoors [10].

3.5. Survey Data

Surveys are employed to collect this type of data by presenting individuals with a series of questions concerning their beliefs, attitudes, behaviors, or experiences. Survey data can be obtained through various means, including online surveys, in-person interviews, and telephone surveys [11].

3.6. Secondary Data

This type of data is collected by third parties for purposes unrelated to the researcher's current study. Secondary data can be obtained from a range of sources, including academic institutions, non-profit organizations, and government agencies [12].

3.7. Meta-Data

This type of data offers information about other types of data, including details on how the data was collected, analyzed, and the conclusions drawn from it. Metadata can be utilized to assist in the interpretation of research findings and assess the quality of research studies [13].

4. DATA ANALYSIS

Recent papers on data analysis, knowledge discovery, and machine learning, including conference proceedings and journals, frequently include statements of this nature. They all share a common concern: how to "make sense" of the vast volumes of data that have been generated, particularly over the past several years [14]. The outcomes of computer-based analysis need to be effectively communicated to users in a comprehensible format. In this context, analysis tools must produce clear and unambiguous results and, in most cases, enable human involvement in the analysis process. Real-world examples of such approaches include representative AI computations that establish an iconic model, such as a decision tree or a set of rules, with a preference for minimal complexity while maintaining high simplicity and accuracy.

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4.1. Intelligent Data Analysis

We must offer a clear definition of what we mean by the term "intelligent data analysis" and clarify its connection to "knowledge discovery in databases" and "data mining." The stages in knowledge discovery in databases (KDD) are often depicted as a systematic process [15]:

• Comprehending the subject matter.

• Creating the dataset and cleaning the data.

• Uncovering data-hidden regularities to create patterns, rules, and other forms of knowledge.

• Data mining (DM), postprocessing of discovered knowledge.

• The utilization of the results is a term used to describe this stage within the broader KDD process.

Intelligent data analysis (IDA) is typically associated with KDD. The relationship between IDA, knowledge discovery in databases, and data mining is illustrated in Fig. (**1**).

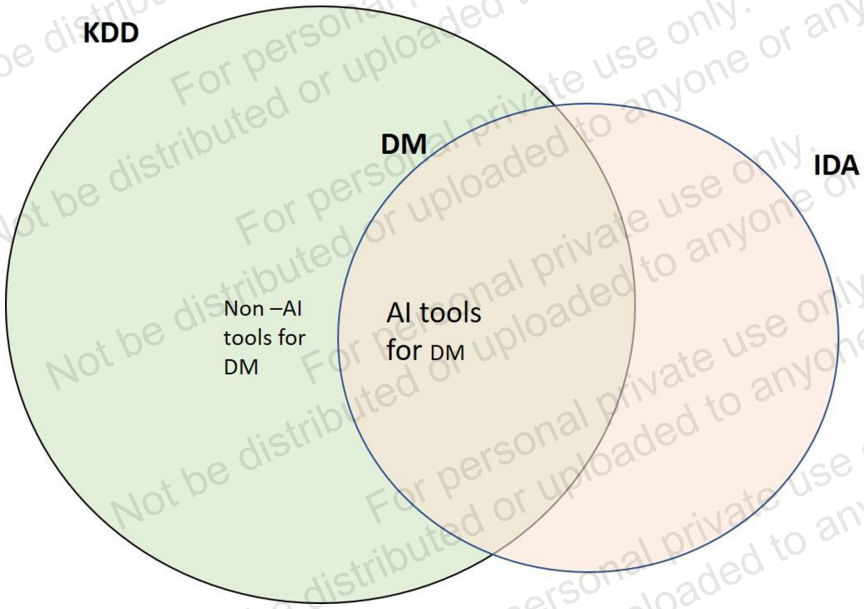

Fig. (1). Relationship of IDA, KDD and DM in data analysis.

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KDD and IDA are intertwined in a significant way. The topic, data analysis, is shared by both fields, as are many of their methods. The main difference between IDA and KDD, as previously stated, is that IDA uses AI tools and methods, whereas KDD uses both AI and non-AI techniques. Another perspective involves the scale of data: KDD typically focuses on extracting information from extremely large datasets, whereas in IDA, datasets can be either large or moderately sized. This also influences the types of data mining tools used: in KDD, data mining tools are predominantly executed in the cluster mode (despite the entire KDD process being intelligent), whereas in IDA, the tools can be used either in a clustered fashion or as intelligent assistants [16].

Two primary perspectives define the significance and need for intelligent data analysis in medicine and pharmacology:

The first significant perspective revolves around the utilization of explicit knowledge-based critical thinking processes (such as inference, prediction, observation, *etc*.) through intelligent analysis of raw data from a specific patient, such as a time series of monitoring data. Much of this data consists of numerical values and is often noisy and incomplete. The objective is to dynamically extract meaningful abstractions, such as summaries of the patient's historical, current, and anticipated future conditions, which can be compared to relevant information (*e.g.*, diagnostic, prognostic, monitoring data, *etc*.). These data analysis techniques are referred to as information deliberation strategies, a term initially coined by Clancey in his now-classic proposal on heuristic classification [17]. In this proposal, these strategies constitute a crucial component of the reasoning process. Recent focus on data abstraction methods has been on the interpretation of temporal data (temporal data abstraction). The primary forms of such abstractions include temporal trends and more intricate temporal patterns. As the primary goal of (temporal) information deliberation techniques is to provide online decision support, their evaluation hinges on performance-based metrics. For example, does a method effectively support diagnostic and prognostic reasoning, and can it accurately predict a trend or value in the near future? In this context, data visualization is pivotal for aiding navigation and surprisingly plays a crucial role in effectively executing critical thinking tasks.

The second aspect involves the generation of novel medical and pharmacological knowledge by mining representative sets of example cases characterized by either numerical or symbolic descriptors. A majority of available datasets are either noisy (containing errors) or incomplete (with missing data). Data mining techniques are employed to extract symbolic knowledge that is both meaningful and comprehensible. The evaluation of these methods includes assessing their

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performance, the quality of the discovered information, and the significance and clarity of the extracted knowledge [18].

4.2. Data Mining

The objective of data mining techniques is to obtain information, preferably in a symbolic form that is meaningful and easy to comprehend. In this context, supervised symbolic learning techniques are commonly employed. For instance, there are powerful tools for inductive learning that can be used to generate meaningful diagnostic and prognostic rules. This framework also encompasses the learning of probabilistic causal networks and symbolic clustering.

Subsymbolic learning and case-based reasoning strategies can also be categorized within the field of data mining [19]. However, the contributions to data mining in this book are limited to symbolic and subsymbolic learning methods. By generalizing from multiple cases, their common objective is to discover the known 1-edge in symbolic or sub-symbolic forms. The representative learning approaches remembered from this book are isolated into propositional (characteristic-based) approaches, inductive rationale programming, and a deterioration way to deal with finding an idea order. The most extensive subset of symbolic learning approaches is the propositional learning approach. Within this subgroup, methods for learning rules from example cases and eliminating noise from example cases are further subdivided. Rules were initially introduced as a primary formalism for symbolically representing knowledge in knowledge-based systems, particularly expert systems. Despite their simplicity and lack of variables, propositional rules are regarded as sufficiently expressive for many real-world applications [20]. The quality of the processed case examples has a significant impact on the viability of rule-learning methods. Commotion in information is an undeniable peculiarity in numerous genuine spaces, in both medicine and pharmacology, noise is a factor that must be acknowledged and managed through computational means. There are two approaches to noise management. either eliminating the noise as a preprocessing step before learning the rules. The majority of the data mining contributions come from symbolic learning methods. Anyway, for a more adjusted image of the significant cutting edge, the incorporation of certain commitments from the sub-symbolic learning region was considered significant.

4.3. Data Abstracting

The identification of temporal abstractions is frequently carried out in a subtle or potentially distributed manner, and it is applicable to both discrete and continuous data streams. Trends, periodic events, and various types of temporal patterns represent valuable forms of temporal abstractions. Visualization techniques can

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also aid in revealing these temporal abstractions [15, 19]. The abstraction can be carried out on a single case, such as a single patient, or a collection of cases. The interpretation of patient data is influenced by relevant contexts in numerous medical fields. For example, the results of certain laboratory assessments can be interpreted differently in two scenarios: "the patient is not receiving any treatment" and "the patient is receiving treatment X" [21]. Regularly occurring events are crucial in medical practice, as symptoms can manifest in repetitive patterns. Periodic occurrences, by definition, involve composite events where successive components are presumed to remain dormant for a specified duration. In general, periodic events constitute a significant type of temporal abstraction. The benefits of distributed processing are well-recognized, and the advent of the Internet has facilitated the rapid growth of telematics tools and applications in handling data. Consequently, the approach to distributed temporal data abstraction aligns with these advancements [22].

An abstract case comprises a set of temporal abstractions derived from a specific case, such as a patient. By synthesizing numerous unique cases, new insights within the specific domain can be generated. From a temporal perspective, visualizing and clustering multiple concrete cases offer an efficient, user-friendly, and computationally economical method for identifying relevant temporal patterns in recent time intervals. These patterns can then be correlated across longer time windows to reveal more generalized temporal patterns. Fig. (**2**) illustrates the types and categories of intelligent data analysis.

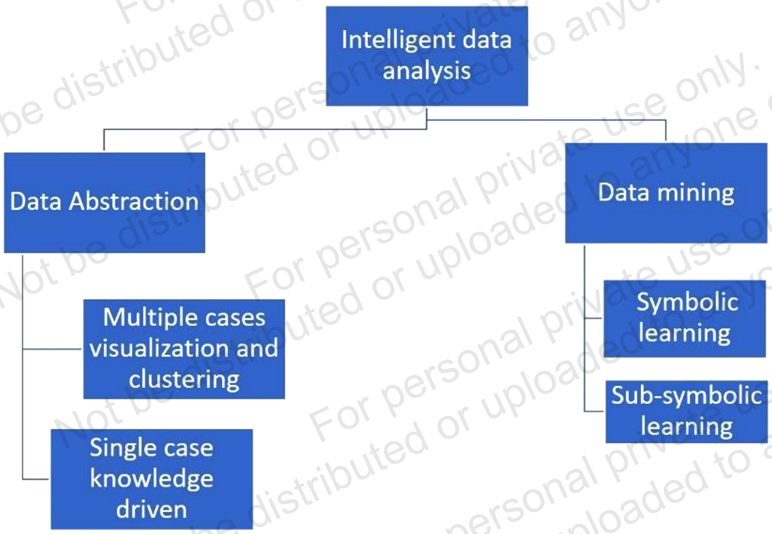

Fig. (2). Types of intelligent data analysis (IDA).

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5. DATA REPRESENTATION

Vast amounts of data are available in nearly all fields of the sciences, yet the analysis of massive, integrated datasets remains challenging. Moreover, because the field of life sciences is highly fragmented, so too are its data and principles. Consequently, integrating data analysis and knowledge gathering across subfields becomes a formidable task. The simultaneous integration of various research perspectives and data types is crucial for achieving a comprehensive understanding of organismal complexity and biological processes [23].

Since the advent of artificial intelligence and the utilization of semantic structures for data representation, diagrams have been employed to formalize how human knowledge is represented. An increasing number of graph-based representation frameworks, such as labeled property graph databases like Neo4j and Resource Description Framework (RDF) triple stores, are known as "knowledge graphs" (KG). General-purpose KGs include Wikidata and the Google Knowledge Graph. In the biomedical sciences, examples include SemMedDB, Hetionet, Implicitome, Ruler Drive, the biological subset of Wikidata, Talked, and KGCOVID19 [24]. A graph with real-world items as nodes and known relationships between those entities as edges may be the most natural definition of KGs, although KGs have been defined in a variety of ways. A KG models the information or "realities" as proclamations, with every assertion being made out of two hubs associated by an edge that portrays their relationship. The statements may include additional features, metadata, and qualifying qualities that further define the properties of nodes and edges and the statement's meaning [25].

Information stored within a Knowledge Graph (KG) can be heterogeneous and diverse, yet it can still be represented in a structured manner within the graph due to the KG's fundamental architecture. The iterative and rapid development of KGs is made possible by representing data as clear connections between fundamental elements. Furthermore, using the graph data structure and a range of inference methods, it is possible to infer additional edges or connections between nodes within the network.

5.1. Software Applications

5.1.1. SPSS (Statistical Package for the Social Sciences)

The statistical software known as SPSS (Statistical Package for the Social Sciences) finds extensive usage in the social sciences, including pharmacy [26]. SPSS can serve various purposes in pharmacy, including but not limited to:

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5.1.1.1. Statistical Descriptions

In pharmacy research, SPSS can be employed for data summarization and description. SPSS is capable of generating descriptive statistics, such as the mean, standard deviation, and frequency.

5.1.1.2. Statistical Inference

In pharmacy research, hypotheses can be tested with SPSS. SPSS can be used to carry out inferential statistics like t-tests, ANOVAs, and regression analyses.

5.1.1.3. Information Perception

Using SPSS, you can create graphs and charts that make it easier to comprehend the pharmacy research data. SPSS can generate histograms, scatter plots, and box plots.

5.1.1.4. Factor Examination

SPSS can be utilized to identify fundamental factors contributing to variations observed in pharmacy data. Factor analysis can assist in reducing data dimensionality and identifying significant variables.

5.1.1.5. Analysis of Clusters

With SPSS, it is possible to group similar observations based on their characteristics. For example, patient subgroups can be identified using cluster analysis, which considers their medication usage patterns.

5.1.1.6. Analyses of Survival

In pharmacy research, SPSS can be used to analyze time-to-event data. For instance, the time it takes for a patient to discontinue medication can be influenced by various factors, and survival analysis within SPSS can aid in identifying and understanding these factors.

In general, SPSS is a versatile tool for interpreting and analyzing data in pharmacy research.

5.1.2. GraphPad Prism

The statistical software package known as GraphPad Prism is commonly used in scientific research, particularly in the fields of biology, medicine, and pharmacy [27]. GraphPad Prism offers a range of methods, including but not limited to:

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5.1.2.1. Analyses of Data

In pharmacy research, data can be analyzed using GraphPad Prism. This includes conducting descriptive statistics, such as calculating the mean and standard deviation, as well as inferential statistics, including t-tests, ANOVA, and regression analyses.

5.1.2.2. Information Perception

GraphPad Prism can be employed to create charts and graphs that aid in visualizing the data collected in pharmacy research. It offers the capability to generate various types of graphs, including scatterplots, bar graphs, and line graphs.

5.1.2.3. A Fitting Curve

Utilizing GraphPad Prism to fit curves to data can assist in identifying trends or patterns within the data. This encompasses fitting various types of curves, including linear, exponential, and sigmoidal curves, among others.

5.1.2.4. Portion Reaction Examination

GraphPad Prism can be used to analyze dose-response data, which is commonly collected in pharmacology research. It facilitates the generation of dose-response curves and the calculation of parameters like EC50 and IC50.

5.1.2.5. Modelling Statistical Data

GraphPad Prism can be utilized to construct statistical models that are employed for making predictions or testing hypotheses. This encompasses survival analysis, meta-analysis, and the development of both linear and nonlinear regression models.

Overall, GraphPad Prism stands out as a robust tool for analyzing and interpreting data in pharmacy research. Its user-friendly interface and comprehensive collection of statistical and graphical capabilities make it a valuable asset. Table **1** provides a list of other state-of-the-art software tools [28 - 34].

5.2. Implementation Application

Applications can play a significant role in enhancing patient care, optimizing processes, and increasing efficiency within the pharmacy industry, especially in drug management and distribution. Some examples of implementation applications in the field of pharmacy include:

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Table 1. Latest software used for data handling of pharmaceutical research.

Sr. No.	Tool/Software	Description	Features
1	Microsoft Excel	A widely used spreadsheet program for organizing and analyzing data.	Data manipulation, formulae, charts/graphs, pivot tables, data validation.
2	Tableau	A business intelligence and data visualization tool for creating interactive dashboards and reports.	Data exploration, visualization, drag-and-drop interface, data blending, geospatial mapping.
3	R	A programming language and environment for statistical computing and graphics.	Data manipulation, statistical analysis, data visualization, machine learning, reproducible research.
4	Python	A general-purpose programming language with a wide range of libraries and frameworks for data analysis.	Data manipulation, statistical analysis, machine learning, data visualization, web scraping.
5	SAS	A suite of software tools for data management, analysis, and visualization.	Data cleaning, statistical analysis, machine learning, data visualization, business intelligence.
6	SPSS	A statistical software package for data analysis and visualization.	Descriptive statistics, inferential statistics, data visualization, predictive analytics, survey research.
7	Power BI	A business analytics service by Microsoft for creating interactive visualizations and reports.	Data exploration, visualization, data modelling, drag-and-drop interface, cloud-based.

5.2.1. EHRs, or Electronic Health Records

Electronic Health Records (EHRs) are digital repositories of patient health information that healthcare providers can access and update. EHRs contribute to improving patient safety by providing accurate and up-to-date medication records, as well as alerts for drug interactions and allergies [35].

5.2.2. Systems for Dispensing Medications

Automated medication dispensing systems can significantly enhance the efficiency of pharmacy operations while reducing medication errors. These systems have the capability to store and dispense medications in accordance with patient orders, in addition to monitoring inventory levels and expiration dates [36].

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5.2.3. Information Systems for Pharmacies

Pharmacy information systems can support the management of pharmacy workflows, encompassing tasks like medication dispensing, inventory management, and billing. These systems can also generate reports and analyses to help identify trends and optimize pharmacy operations [37].

5.2.4. Apps for Mobile Devices

Patients can leverage mobile applications to access medication information, request prescription refills, and receive medication reminders. Furthermore, healthcare providers can use these applications to communicate with other providers and access patient health records [38].

5.2.5. Tele Pharmacy

Technology plays a vital role in tele-pharmacy, enabling services such as medication management and counseling to be provided remotely. This is particularly beneficial for patients residing in rural or underserved areas who may lack access to a nearby pharmacy [39].

In summary, the implementation of applications in the pharmacy setting can contribute to enhancing patient care, increasing efficiency, and reducing medication errors. These applications can improve various aspects of pharmacy operations, including medication dispensing, inventory management, and patient education.

5.3. Examples of Implementation

Several instances of implementations adopted in pharmacies have been widely embraced to improve patient outcomes and streamline pharmacy operations. Some examples include:

5.3.1. Electronic Recommending

Electronic prescribing, or e-prescribing, enables healthcare providers to electronically transmit prescriptions directly to the pharmacy [35]. This can help reduce medication errors and enhance patient safety by providing accurate and up-to-date medication information.

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5.3.2. Automating Pharmacies

Automated medication dispensing cabinets and dispensing robots, for instance, can aid pharmacies in reducing medication errors, enhancing productivity, and conserving staff time [36].

5.3.3. The Clinical Choice was Emotionally Supportive Networks

Clinical decision support systems offer healthcare providers alerts, reminders, and real-time clinical information to enhance patient care. These systems may also provide drug information, dosage recommendations, and clinical guidelines, all aimed at improving patient outcomes [37].

5.3.4. Management of Medical Treatment

Medication therapy management (MTM) is a comprehensive approach to managing medication use and enhancing patient outcomes. It includes services such as medication reviews, drug therapy monitoring, patient education, and support for medication adherence, all aimed at optimizing patient care [38].

5.3.5. Purpose of Care Testing

Point-of-care testing is performed at the patient's location, such as in a pharmacy, and enables healthcare providers to conduct diagnostic tests. This approach, which leads to quicker treatment and faster results, can contribute to enhanced patient outcomes [39].

CONCLUSION

Since the turn of the century, pharmaceutical research has made substantial progress and has greatly contributed to society. However, the effective utilization of high-quality research data remains a significant challenge in both research and its application in the medical field. This article has explored both traditional and contemporary software developments in the field of medical data management. Such software can be valuable not only for managing research data but also for routine hospital operations. The historical section delved into the early manual data management practices and the subsequent evolution of software solutions. Additionally, various categories and subcategories of data types were explained. We aimed to encompass its implementation in both research and medical practices, emphasizing the proper utilization of data management software.

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Engineering Materials

Sabu Thomas
Poushali Das
Sayan Ganguly *Editors*

Quantum Dots Based Nanocomposites

Design, Fabrication and Emerging Applications

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This series provides topical information on innovative, structural and functional materials and composites with applications in optical, electrical, mechanical, civil, aeronautical, medical, bio- and nano-engineering. The individual volumes are complete, comprehensive monographs covering the structure, properties, manufacturing process and applications of these materials. This multidisciplinary series is devoted to professionals, students and all those interested in the latest developments in the Materials Science field, that look for a carefully selected collection of high quality review articles on their respective field of expertise.

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Editors

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Design, Fabrication and Emerging Applications

Springer

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Preface

Enter the captivating world of nanocomposites that leverage quantum dots! The book, named "Quantum Dots Based Nanocomposites: Design, Fabrication and Emerging Applications," provides a thorough investigation of the advanced topic that combines quantum research and nanotechnology. At the vanguard of scientific progress, the incorporation of quantum dots into nanocomposite materials holds the potential to profoundly transform multiple industries and unlock unparalleled opportunities.

The impetus for this work arises from the notable advancements observed in the synthesis, design and application of quantum dots in recent times. Quantum dots, due to their distinct electrical and optical properties that vary based on their size, have become essential components for developing innovative nanocomposites. These nanocomposites possess synergistic features that surpass the abilities of their individual components, resulting in improved performance and multifunctionality.

This volume explores the complexities of nanocomposites based on quantum dots. The book is organised in a manner that aims to offer a thorough comprehension of the principles of design, processes of manufacturing and the wide range of new applications in various sectors such as electronics, photonics, energy, medicine and others. Every chapter is meticulously designed to explore distinct facets, providing a combination of theoretical underpinnings and practical observations derived from recent research endeavours.

The initial segment of the book presents the fundamental notions of quantum dots, their distinctive quantum mechanical characteristics and the underlying rules governing their incorporation into nanocomposite structures. The following chapters examine several fabrication methods, investigating the intricacies of building these nanocomposites at the nanoscale to achieve the best possible performance. The book covers a wide range of techniques used in the production of nanocomposites based on quantum dots, including solution-based approaches and sophisticated nanofabrication processes.

The book's second portion centres on the varied applications that have arisen from the combination of quantum dots with different matrices. The range of possibilities, from sophisticated electrical gadgets to effective energy conversion systems and precise drug delivery in medicine, is extensive and continuously developing. The

investigation of various applications demonstrates the adaptability and profound capacity for change of nanocomposites based on quantum dots.

As the editors of this book, we have had the honour of working together with prominent scholars and specialists in the subject, who have each provided their distinct viewpoints and specialised knowledge. The collaborative endeavour seeks to furnish an all-encompassing repository for researchers, scientists, engineers, and students who are enthusiastic to delve into the forefront of this swiftly advancing domain.

This book aims to be a beneficial reference for anybody interested in exploring the fascinating field of nanocomposites based on quantum dots. As we explore the complex aspects of design, fabrication and developing applications, we welcome you to join us in understanding the secrets and harnessing the immense possibilities that this sector offers for the future.

Enjoy your reading and exploration!

Kerala, India Sabu Thomas
Hamilton, Canada Poushali Das
Waterloo, Canada Sayan Ganguly

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Introduction to Quantum Dots Based Nanostructures

Sayan Ganguly

Abstract This chapter provides an overview of the exciting new area of quantum dots composites, including information on their preparation, characteristics, and potential uses. Nanosized semiconductor particles are known as quantum dots exhibit size-dependent properties that make them a promising candidate for use in different composites. The chapter discusses several synthesis methods, emphasising the need of exact control over quantum dot size and composition. Quantum dot composites are discussed, along with their many potential uses in fields like optoelectronics, photovoltaic, biomedicine, and sensing. Improved optical, electrical, and mechanical qualities are only a few of the ways in which these composites could revolutionise several fields of technology. This section lays the groundwork for future study and development in this exciting topic.

Keywords Quantum dots composites · Semiconductor · Optoelectronics · Photovoltaics · Biomedicine · Sensing

1 Introduction

In 2004, Xu et al. purified SWCNTs to create luminous carbon nanomaterials [1]. Sun et al. later in 2006 developed fluorescent (FL) carbon NPs, dubbed carbon dots, in both a solvent-dispersed and a solid-extracted form [2]. Many studies have since been conducted to learn more about CDs from the perspectives of synthesis, characterisation, and application. Carbon dots (CDs) [3, 4], Carbon quantum dots (CQDs) [5, 6], Carbon nanodots (CNDs) [7], Graphene quantum dots (GQDs) [8, 9], and Polymer dots (PDs) [10] are all names that have been offered for these nanomaterials. Fluorescence is an important tool in the life sciences, where it is utilised to investigate cellular interactions and the dynamics between biomolecules. To label, detect, and visualise the bio-targets, several fluorophores are created for this method.

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Small compounds, proteins, or quantum dots (QDs) are used as these fluorophores. Quantum dots (QDs) are semiconductor nanoparticles with diameters between 2 and 10 nm. The atoms that make them up are often found in either group II–VI or group III–V of the periodic table. QDs exhibit fluorescence in a different way, by quantizing their semiconductor energy level less than their nanometer sized radius, whereas tiny molecules' fluorescence contributes to delocalized electrons that can leap a band and stabilise the energy absorbed. There are primarily three reasons why QDs have drawn more great attention from biologists and chemists than tiny fluorescent molecules and protein fluorophores. First, the wavelength of the band-edge adsorption and fluorescence emission of QDs can be changed systematically by varying their size. Secondly, the photoluminescence spectra of QDs may be observed by a single excitation source over a broad wavelength range, from the visible to the near-infrared. Third, QDs can be used for dynamic imaging of living cells because to their long luminous life and great photostability. Qdots can be broken down into two major classes: elemental and compound. Nanostructured materials based on compound semiconductors are discussed, together with their optoelectronic and optical properties, and potential multimodal applications are highlighted.

There has been a rise in interest in studying composites made from CDs and other functional molecules/materials. The limitations of pure CDs can be greatly reduced and their usefulness expanded by using composites. CDs' many surface functional groups make it simple for them to build composites with other materials. Polymers [11], inorganic nanostructures [12], and biomaterials [13] have all been used successfully in the creation of CDs composites to date. Composites of polymers with fullerene, carbon nanofibers, carbon nanotubes, and graphene-based materials are also possible [14–16]. CDs may include polymers to make the composites with little drawbacks and maximal benefits, unlike other carbon nanostructures. Composites made from CDs have been created by incorporating the materials into polymer matrices, polymeric gel, and molecularly imprinted polymers [17]. CDs can interact strongly with polymers due to their tiny size and vast number of surface functional groups, allowing for homogenous dispersion within the polymers. These composites feature advantageous properties such as low toxicity, low photobleaching, high chemical stability, low absorption, good functionalization, and photobleaching resistance [18]. CD/polymer composites are more hydrophobic, durable, flexible, and strong than their individual components [19]. Some polymers with desirable properties, including as self-healing, increased mechanical performance, shape memory, anticorrosion, stimuli-responsiveness, and electrochemical characteristics, may be made available through CD coating. Anticounterfeit, ultraviolet (UV) shields, optoelectronic devices, solar cells, supercapacitors, solid-state optical films, catalysts, and sensors are just some of the possible uses for CD/polymer composites [20].

The revolutionary field of quantum dot-based composites, on the cutting edge of nanotechnology, is introduced in Quantum Dot-Based Composites. Quantum dots' size-dependent optoelectronic qualities are highlighted in this chapter as we investigate their fundamental properties and fabrication procedures. Composites made of quantum dots are revealed to be transformative in a variety of fields. We dive into their significance in electronics, where they enhance transistor efficiency, improve LED

and solar cell performance, and pave the path for high-efficiency devices. Their incorporation with photonics materials makes possible the development of technologies like tunable lasers, high-resolution screens, and cutting-edge imaging equipment. Quantum dot-based composites have several uses outside of technology, including in health (improvements in medication delivery, imaging, and diagnostics) and the environment (helping with clean energy production and environmental monitoring) and the energy sector (facilitating clean energy production). We recognise the difficulties, including toxicity and scalability, but also show glimpses of a bright future in which quantum dot composites may catalyse astonishing discoveries across industries. Case studies from the real world illustrate their current influence and provide readers a full picture of this revolutionary technology's potential to alter the field of materials science.

2 Synthesis Methods for QD/Polymer Composites

Both top-down and bottom-up synthesis approaches are viable options for the preparation of QDs. Laser ablation, electrochemical oxidation, chemical oxidation, arc discharge, and ultrasonic treatment are some of the physical and chemical processes that are used in top-down techniques [21]. These processes are used to destroy larger carbon compounds. The bottom-up procedures, on the other hand, are characterised by the transformation of tiny carbon molecules into QDs through the use of a chemical reaction, such as pyrolysis, plasma treatment, solvothermal therapy, hydrothermal treatment, microwave treatment, or the template route. Treatments such as hydrothermal, solvothermal, microwave, pyrolysis, and ultrasonic synthesis have been utilised in the process of preparing QDs prior to their incorporation with polymers. In the meantime, QD/polymer composites have been prepared using a variety of methods, such as stirring, sol–gel, drop casting, conventional solution casting, in situ chemical polymerization, reverse microemulsion polymerization, interfacial polymerization, cross-linking reaction, photopolymerization, polymer-assisted self-assembly, Schiff base reaction, bulk polymerization, electrospinning, thermal treatment, hydrothermal treatment, nonsolvent induced According to our review of the relevant published research, the hydrothermal treatment has been the one used most frequently for the manufacture of QDs for composites. The names of composites as well as the characteristics of the synthesis processes for QDs and QD/polymer composites are listed in Table 1. This section provides an overview of the synthesis processes described in the previous section for the production of QD/polymer composites.

Table 1 Different types of CDs based composites and their synthesis methods

Composite	Method	Temperature (°C)	References
PVA/CDs	Hydrothermal	260	[22]
MIPs/CDs	Hydrothermal	200	[23]
PVA/CDs	Hydrothermal	160	[24]
PPy/CDs	Hydrothermal	230	[25]
PANI/CDs	Hydrothermal	230	[25]
CDs/Silica	Hydrothermal	180	[26]
GQDs/polyimide	Hydrothermal	120	[27]
CDs/polyethylenimine/polyethersulfone	Hydrothermal	180	[28]
CDs/thermoplastic polyurethane	Hydrothermal	170	[29]
CDs/Fe_3O_4	Solvothermal	240	[30]
CDs/polyethyleneimine	Solvothermal	150	[31]

3 Properties of QDs

Nano-materials, including quantum dots, have been broken down into three different nanosystems and one bulk system. In other words, as n-dimensional materials with (3-n)-angles of quantum confinement, such as bulk mate-rials (n = 3), quantum wells (thin films; n = 2), quantum wires (nanotubes; n = 1), and quantum dots (nanoparticles; n = 0). Due to their distinct photophysical and electrical properties after photon excitation, quantum dots have found widespread application in a variety of devices and applications, including but not limited to light-emitting diodes (LEDs), solar cells, bioimaging, sensors, displays, quantum communications, and lasers [32]. The phenomenon of photoemission in quantum dots, which is influenced by the size of the dots, has been explained by considering the concepts of quantum confinement and quantum size effects. This explanation has been achieved by employing a square-well potential model. The initial phenomenon pertains to the condition of electrons that are contained within the exciton Bohr radius. Consequently, the freedom of an electron becomes significantly restricted within a quantum dot, resulting in a larger bandgap compared to a bulk system [33]. Quantum dot composites (e.g., core-shell organic-inorganic hybrids) and encapsulation can stabilise and improve photoluminescence quantum yields (s) due to the QDs' enhanced quantum confinement effects. For instance, in comparison to a core-type QD, the photo-emission of a core/shell-type QD is higher because the wide-bandgap inorganic shell serves to remove surface imperfections and dangling bonds. In addition, hetero-junctions can be formed between the core QDs and other inorganic materials with similar lattice constants and crystalline structures during the synthesis of core/shell-type QDs and encapsulated QDs, thereby providing further physical stabilisation.

Applications in electronics, photonics, and even medicine have resulted from these qualities. In this article, we will explain the significance of some of the most important physical features of quantum dots.

3.1 Size-Dependent Optical Properties

Semiconductor crystals on the nanoscale, such as cadmium selenide (CdSe), lead sulphide (PbS), or indium arsenide (InAs), are what we call quantum dots. The bandgap and, by extension, the optical properties of a quantum dot are strongly dependent on their size. Bigger bandgaps in smaller quantum dots lead to higher-energy emissions, while smaller bandgaps in bigger quantum dots lead to lower-energy emissions. This size-dependent behaviour is called the quantum confinement effect, and it allows scientists to tailor quantum dots for uses like LEDs and displays based on quantum mechanics.

3.2 Exceptional Photostability

Quantum dots have remarkable photostability, which allows them to maintain their light emission over extended periods of time without degrading significantly. This quality is especially useful in biological imaging and fluorescence microscopy, where quantum dots can be employed as highly efficient and stable probes. Quantum dots are superior to conventional organic dyes for researching biological processes due to their resistance to photobleaching.

3.3 High Quantum Yields

The efficiency with which quantum dots emit light, or their "quantum yield," is quite high. They may emit a considerable amount of absorbed photons as fluorescence, making them advantageous for applications such as biological labelling and LED technology. Surface states have a significant role in increasing radiative recombination processes, which contributes to quantum dots' high quantum yields as a result of their unusual electronic structure.

3.4 Tunable Emission Wavelength

The ability to adjust the quantum dot's emission wavelength is a major benefit. Scientists are able to precisely tune the colour of produced light by manipulating the quantum dots' size and composition. In multiplexed biological imaging, where distinct colours of quantum dots are utilised to track different cellular components, this characteristic is leveraged to create colourful and high-quality displays.

3.5 Broad Absorption Spectrum

Quantum dots can yield in light from a wide variety of wavelengths according to their large absorption spectrum. Because of this, quantum dots have found a particularly valuable application in solar cell technology, where they can be incorporated into photovoltaic devices to efficiently harvest sunlight. Quantum dots of varying sizes and compositions can be used to tailor the solar cell's absorption spectrum and, in turn, the cell's ability to convert light into usable energy.

3.6 Size-Dependent Electrical Properties

Quantum dots display size-dependent behaviour in both their optical and electrical properties. Quantum dots can function as quantum wells, wires, or dots, depending on their size and shape. The capacity to fine-tune the electrical behaviours of quantum dots is particularly useful in applications involving semiconductor devices like transistors and sensors.

3.7 Quantum Confinement Effects

The quantum confinement effect, coming from the reduced dimensionality of quantum dots, leads to discrete energy levels. Because of their distinct energy levels, quantum dots exhibit characteristically sharp absorption and emission peaks. This phenomenon stands in stark contrast to the continuous energy levels present in bulk materials. The novel optical and electrical features of quantum dots are based on their quantum confinement, which has implications for their employment in a wide range of technologies.

Quantum dots are a type of nanomaterial with many interesting physical properties. These include optical properties that vary with size, high quantum yields, a broad absorption spectrum, tunable emission wavelengths, and quantum confinement effects. Due to their unique characteristics, quantum dots have found use in a wide variety of technological contexts, including electronic displays, medical imaging, solar cells, and semiconductors. Their versatility as a platform for targeted engineering keeps nanotechnology at the forefront of scientific inquiry and development, pointing to promising new frontiers in the area.

4 Applications

4.1 Solar Energy Conversion

Using solar energy to generate electricity or hydrogen for fuel requires the application of cheap materials that can capture photon energy. There are currently two technologies that can harvest and convert solar energy: (i) PEC cells and (ii) photovoltaic solar cells. Heterostructures with an appropriate bandgap alignment and appropriate interfaces would be beneficial for high efficient charge separation in solar cells. QDs in semiconductor heterostructures can facilitate fast charge transfer. Heterostructures made out of QDs consist of two or more parts separated by an interface; these parts are either donors or acceptors. Donor materials are photoexcited to create electrons and holes, which are then transferred to the acceptor across the junction. If you're looking for a donor material, make sure it has a wide bandgap and a high absorption coefficient for the solar spectrum. In addition, the donor and acceptor conduction bands should be in the right place with respect to one another. The acceptor would serve as a conduit for charge carriers to travel from the donor to the electrode's conductive covering [34]. PECs, films containing QDs submerged in an electrolyte, and QD-sensitized solar cells are only a few of the QD-based solar cell topologies that have been proposed. The bandgap of QD-based solar cells can be adjusted, which is an advantage. The QD-based solar cells can overcome the Shockley-Queisser limit. Many semiconductor QDs have been synthesised for application in the sensitization of wide bandgap semiconductors. These materials include CdSe, CdS, PbS, InAs, etc. Because of their size, the QDs can only absorb light within a specific range of the sun's spectrum [35]. The creation of solar cells that utilise QDs and carbon nanostructures is only getting started. The next generation of solar cells may result from investigations investigating the use of these materials [36].

4.2 Photoelectrochemical Water Splitting

A polymer electrolyte membrane (PEC) cell is a type of solar cell that may be used to either directly produce electricity or electrolyze water into oxygen and hydrogen. Hydrogen is created by the PEC water splitting process, which uses solar energy to create chemical energy. It has been proposed that this cell could be used to convert solar energy into usable hydrogen fuel by shining light on a photoanode. PEC photoanodes typically consist of TiO2, ZnO, and WO3, which are all transition metal oxides. These oxides' photoanodes have poor performance because to low light absorption and rapid recombination. However, there have been some advancements in terms of light absorption and the decreased rate of recombination by employing semiconductor QDs. Semiconductor QDs have the disadvantages of being poisonous, expensive to produce, and unstable when used in photoanodes [37]. CQDs can be

utilised in PEC photoanodes in place of semiconductor QDs, thereby reducing toxicity issues while increasing stability and production cost. The ability to use CQDs as a photosensitizer is enhanced by their broad spectrum absorption. In CQDs, the excited electron relaxes to the ground state via a gradual process of fluorescence or phosphorescence rather than thermal relaxation. As a result, the electron can be transported from the sensitizer to the contacting acceptor. Under irradiation from a 300 W Xe lamp with a L42 cutoff filter (using this filter causes just the visible and NIR portion of the spectrum to be transmitted), hydrogen generation is only detectable for the photoanode sensitised with CQDs. This demonstrates that the use of CQDs in the photoanode is necessary for hydrogen evolution to occur [38]. CQDs-sensitized TiO_2 was employed for water splitting. When exposed to visible light, the built photoanode showed up to five times the activity of TiO_2 without CQDs. Visible light irradiation of the CQDs causes a dramatic increase in their activity. In PEC, CQDs/TiO_2 photoanodes exhibit enhanced light absorption in proportion to the CQDs concentration [37]. In a PEC cell for water oxidation, N-doped CQDs have been employed for a hematite nanorods array on a Ti sheet. N-CDs@α-Fe_2O_3/Ti photoanode had a 3.4-fold increase in photocurrent density over pure α-Fe_2O_3/Ti. N-doped CQDs have an up-conversion fluorescent feature that allows them to absorb light at longer wavelengths and emit light at shorter wavelengths. Excitation of α-Fe_2O_3 and formation of electron-hole pairs can also occur in response to irradiation with a shorter wavelength [39].

4.3 Agriculture Sector

Pre-harvest (growth, production, and disease resistance) and post-harvest (food preservation and safety) are two areas where agriculture has benefited from QDs [40]. CDs play a part in the pre-harvest process because they act as nano-bio-fertilizers, increasing the efficiency with which plants convert sunlight into usable energy by activating additional photosynthetic genes like PsbP and PsiK [41]. QDs have been observed to potentiate growth regulators, boost cellulose and protein content, and improve germination rates [42]. Considering post-harvest, QDs show bacterial inhibitory effects. Fan et al. discovered that CDs made from kelp significantly reduced the number of Staphylococcus aureus and Escherichia coli on cucumbers during storage [43]. Mangoes were found to maintain their high quality even after being stored for an extended period of time when covered with QDs and chitosan, which was proven to prevent water loss and sugar acid conversion [44]. Therefore, it would be of great interest to design a programme for the widespread use of QDs in agricultural fertilisers and materials for coating agricultural goods, and to investigate their toxicity along the food chain (effects on soil, plants, animals, and humans). Plants have also benefited from QD-based genetic engineering. Schwartz and coworkers synthesised CDs from polyethyleneimine using the solvothermal technique. Tomatoes from the Nicotiana benthamiana 16C line were successfully dosed with CD/siRNA, resulting in gene silencing [45].

4.4 Anti-Counterfeit and Security

Forensic medicine makes use of CDs since it is a field that gives scientific proof in the fight against counterfeiting [4]. Orange-emitting CDs made from the chemical rhodamine B were used to quickly and efficiently visualise latent fingerprints after four months under ultraviolet (UV) light and in poor settings, such as curved and coloured surfaces, according to a prior study [46]. Moreover, CDs have been used in anti-counterfeit systems to identify counterfeit bills. Triple-mode photoluminescence when activated with 365 nm UV light was displayed by CD-based printable ink produced from luminescent m-phenylenediamine and poly(vinyl alcohol) to identify counterfeit banknotes [47]. CDs have been the subject of numerous drug/narcotic/explosive chemical detection tests [48]. When it comes to finding fakes, CDs are a need. So, it's important to equip counterfeit investigators with portable carbon-dot devices or sensors that can withstand high temperatures and moisture. After combining eco-friendly fluorescent CDs (made from turtle shell), researchers recently built colloid photonic crystal (CPC)/CD beads using P(St-MMA-AA) microspheres; the resulting fluorescent CPC/CD patterned films were used as a multi-signal anti-counterfeiting device [49].

5 Conclusions

As this chapter draws to a close, a thorough introduction to a fascinating and quickly developing area of materials science and nanotechnology has been presented. Due to their size-dependent features, quantum dots have enabled intriguing new possibilities in the development of high-performance composites for use in a wide variety of fields. In this chapter, we have looked at the basics of quantum dots and how they can be used to improve the properties of composites, such as their optical, electrical, and mechanical qualities. Colloidal, epitaxial, and organic–inorganic hybrid production methods for quantum dots were explored, and the need of having fine-grained control over their size and composition was emphasised. Additional uses of quantum dots composites, such as in optoelectronics, photovoltaics, biomedical imaging, and sensors, were explored in this chapter. We discovered that these nanocomposites have the potential to completely transform technology in various fields due to their better performance.

In conclusion, the investigation of quantum dots composites is not only interesting from a purely scientific viewpoint, but also has tremendous potential in a variety of real-world settings. We can look forward to significant advancements that will define the future of materials science and technology as researchers continue to investigate and create in this area. This chapter has set the foundation for further investigation and experimentation in the field of quantum dots composites, opening the door for new discoveries and advancements.

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Discovery and History of Quantum Dots

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Abstract Quantum dots are a type of semiconducting material that possesses distinctive electronic and optical characteristics at the nanoscale level. As a result, they have found extensive utility in a diverse array of scientific and technological domains. The present chapter provides a historical account of quantum dots, beginning with their initial discovery in the 1980s and extending to contemporary times. The chapter provides an overview of the different techniques employed in the synthesis and fabrication of quantum dots, which include colloidal synthesis, vapor-phase synthesis, and epitaxial growth. The text delineates the various categories of quantum dots, including core-shell and alloyed quantum dots, and elucidates how their characteristics can be adjusted to cater to particular use cases. Moreover, the chapter explores the diverse implementations of quantum dots in domains such as

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photovoltaic technology, illumination, medical visualisation, and quantum information processing. The text also addresses the potential hazards linked to quantum dots, such as apprehensions regarding toxicity and ecological consequences.

1 Introduction

1.1 What Are Quantum Dots?

Quantum dots are incredibly small nanocrystals with sizes in the region of a few nanometers that are formed of semiconducting materials. They can be compared to synthetic atoms because of the distinct electrical and optical characteristics they display that are not present in their bulk counterparts [1]. Due to their tiny size, which causes the material's electrical characteristics to become quantized, quantum dots have peculiar properties [2]. Particularly, the quantization of the electronic energy levels occurs when a quantum dot is created and its electrons are constrained within a constrained area of space [3]. Due to this, discrete energy levels are created, and these levels are governed by the quantum dot's size, composition, and shape [4]. These energy levels are known as the "quantum confinement effect," and they give birth to a variety of intriguing features that may be tweaked and controlled by altering the quantum dot's size and make-up [5]. Optoelectronics is one of the most promising fields in which quantum dots can be used. In example, photovoltaic cells and highly efficient light-emitting diodes (LEDs) can be produced using quantum dots [6–8]. The colour of the light emitted by the LED may be precisely modified due to the controllable quantum dot size [9, 10]. Quantum dots can also be employed as down-converters to boost solar cells' efficiency by expanding the quantity of light that can be absorbed. Biomedicine is a promising area for using quantum dots. Quantum dots are fluorescent probes that can be utilised for imaging and disease diagnostics [11–13]. Due to their tiny size, quantum dots can enter cells and tissues and produce high-resolution photographs of biological structures. Quantum dots can also be functionalized with particular targeting molecules to bind to certain cells or tissues with a specific preference, enabling tailored drug administration [14]. Quantum dots are an exciting area of study that could revolutionise a variety of industries, including optoelectronics, healthcare, and energy [15–22]. Due to their special qualities, such as the quantum confinement effect, quantum dots are highly adaptable materials that can be tweaked and controlled to fit various needs [23]. They will therefore probably continue to play a significant role in many fields of science and technology going forward.

The distinctive characteristics and potential uses of quantum dots render them significant in both technological and research domains [24]. Nanoparticles of semiconductors can be accurately designed to exhibit distinct electronic and optical characteristics, rendering them advantageous in various domains such as optoelectronics,

biomedicine, and energy [14]. Quantum dots possess the potential to yield light-emitting diodes (LEDs) and photovoltaic cells with exceptional efficiency in the field of optoelectronics. Quantum dots possess the potential to enhance colour accuracy and diminish power consumption in displays and lighting applications due to their capability to emit light at highly specific wavelengths [25]. Furthermore, quantum dots possess the potential to serve as down-converters, thereby enhancing the efficacy of photovoltaic cells through the amplification of light absorption [26]. Quantum dots have been identified as potential fluorescent probes for imaging and diagnosis of diverse diseases in the field of biomedicine [27]. The diminutive dimensions of quantum dots facilitate their infiltration into tissues and cells, thereby affording high-fidelity visual representations of biological structures [28–30]. Furthermore, quantum dots possess the ability to be functionalized with particular targeting molecules, which can facilitate the selective binding to specific cells or tissues. This feature allows for the possibility of targeted drug delivery [16, 20, 31]. Quantum dots possess the potential to enhance the efficacy of solar cells by augmenting the extent of light absorption in the field of energy. Researchers have the ability to produce materials that can absorb light across a wide range of wavelengths by adjusting the size and composition of quantum dots [32]. Quantum dots hold significant importance in the realm of fundamental research in physics and materials science, in addition to their specific applications [33]. Quantum dots can be employed by researchers to investigate the conduct of electrons within a restricted area, thereby facilitating an enhanced comprehension of quantum mechanics and the emergence of novel materials and technologies [34]. Quantum dots possess distinctive characteristics and hold significant potential for utilisation in diverse domains, rendering them crucial in both technological and research contexts [35]. Nanoparticles possess the capability to bring about a significant transformation in domains such as optoelectronics, biomedicine, and energy. It is highly probable that they will persist in playing a crucial role in various fields of science and technology in the forthcoming years.

1.2 Early History of Quantum Dots

The inception of quantum dots can be traced back to the early 1980s, when the Russian physicist Alexei Ekimov and the American physicist Louis E. Brus conducted groundbreaking research in this field. In the year 1982, Ekimov made a discovery regarding small semiconductor crystals that were referred to as “zero-dimensional” entities. These crystals displayed quantum confinement effects that were not observable in bulk semiconductors [36]. In 1983, Brus made independent discoveries that yielded similar effects and subsequently introduced the term “quantum dots” to refer to these minute nanocrystals. The research conducted by Brus was centred on the optical characteristics of said materials, demonstrating that they discharged light at distinct wavelengths which were contingent on their dimensions. During the decades of 1980s and 1990s, scholars conducted further investigations on the characteristics of quantum dots and devised novel approaches for their synthesis and

manipulation. In 1994, a group of researchers from Bell Labs conducted a demonstration that showcased the potential of quantum dots in the development of light-emitting diodes (LEDs) with superior efficiency. This breakthrough discovery paved the way for novel applications in the field of optoelectronics. Subsequent to that time, scholars have achieved noteworthy progress in the production and analysis of quantum dots, resulting in novel implementations in the domains of biomedicine, energy, and other related areas [2]. Quantum dots represent a burgeoning field of study that is currently experiencing rapid growth, owing to their numerous potential applications and substantial commercial appeal [37–39].

Quantum mechanics underpins quantum confinement effects in quantum dots. Knowing electron behaviour in quantum dots requires knowing the wave-particle duality of matter and energy quantization in restricted systems. Electrons travel freely in a three-dimensional lattice structure in bulk semiconductor materials. Electrons can occupy any energy level in these continuous materials. However, nanoscale semiconductor materials restrict electron movement in all three dimensions. Electron energy levels are quantized by confinement, depending on the quantum dot's size and shape [40]. Quantized energy levels in quantum dots provide them unique features not found in bulk semiconductors. Quantum dots produce light at specified wavelengths depending on their size and shape, hence their emission spectra are crisp and narrow. Quantum dots are potential for LEDs and solar cells due to their emission spectrum tenability [41, 42]. The effective mass approximation and k-p approach are used to calculate quantum dot electron energy levels and wave functions. These models take into account quantum confinement, semiconductor crystal structure, and composition. Quantum physics predicts that electron energy levels become quantized in restricted systems, which underlies quantum confinement effects in quantum dots [17, 43, 44]. Quantization of energy levels in quantum dots produces unique electrical and optical features that have many uses in science and technology [45, 46].

The investigation of size-dependent properties of nanocrystals was initiated in the 1980s through early experimental studies. The phenomenon of the shift in absorption and emission spectra towards higher energies was observed by researchers in certain semiconductor nanocrystals, as the size of the nanocrystals decreased. The observed blue-shift in the spectra has been ascribed to the quantum confinement effect, which emerges from the confinement of electrons and holes within a limited volume of the nanocrystal [47]. The phenomenon in question leads to the quantization of energy levels of both electrons and holes, thereby causing alterations in the optical and electronic characteristics of the nanocrystals that are dependent on their size [48]. The initial investigations initiated a novel area of inquiry regarding the characteristics and prospective uses of nanocrystals. Apart from the blue-shifted absorption and emission spectra, the scholars also noted alterations in various characteristics of nanocrystals, including their magnetic behaviour and conductivity, which were dependent on their size. As particle size decreases in certain materials, such as gold and silver, the melting point exhibits a corresponding decrease [49]. The observed phenomenon, commonly referred to as "melting point depression", can be attributed to the heightened surface area-to-volume ratio of the particles. This characteristic renders the particles more vulnerable to surface melting and thermal

fluctuations [50]. Size-dependent properties of nanocrystals have been investigated, including their mechanical characteristics, such as hardness and ductility, as well as their catalytic activity in chemical reactions. The impact of the morphology and constitution of nanocrystals on their characteristics, as well as their interplay with other substances and biological systems, has been examined by scholars. In general, the initial experimental investigations concerning the size-dependent characteristics of nanocrystals have initiated a novel area of inquiry into the attributes and prospective uses of materials at the nanoscale. Currently, the investigation of nanocrystals and other nanomaterials is a swiftly expanding field of study with numerous potential applications in domains such as electronics, energy, biomedicine, and other related fields.

1.3 First Synthesis of Quantum Dots

Louis E. Brus and his team accomplished the initial prosperous amalgamation of quantum dots in 1984. The researchers employed solution-phase methodologies to produce colloidal quantum dots composed of semiconductors, specifically CdSe, CdTe, and CdS [51]. The process of synthesis entailed the introduction of precursors, namely cadmium and selenium, into a heated solution of a coordinating solvent, such as tri-n-octylphosphine oxide (TOPO) and trioctylphosphine (TOP). Subsequent to the reaction, a coordinating ligand, namely hexadecylamine (HDA), was introduced to serve as a capping agent with the purpose of regulating the dimensions and morphology of the quantum dots [52]. The quantum dots obtained exhibited a high degree of uniformity in size, superior quantum efficiency, and a robust luminescent property, rendering them a desirable candidate for a diverse array of applications. The triumph of this particular method of synthesis has paved the way for further exploration into the synthesis and characteristics of colloidal quantum dots. Currently, solution-phase synthesis is a frequently employed technique for the production of quantum dots, with numerous modifications and advancements implemented over time to enhance regulation of the quantum dots' size, shape, and characteristics. Brief explanation of the synthesis technique.

2 Key Contributions from Early Researchers

The initial investigators of quantum dots made significant advancements that facilitated their current utilisation in various domains. The identification of the quantum confinement effect stands out as a significant contribution. During the initial years of the 1980s, Alexei Ekimov and Louis E. Brus conducted research and made observations that revealed the absorption and emission spectra of specific semiconductor nanocrystals underwent a shift towards higher energies with a decrease in the size of the nanocrystals. The observed blue-shifted spectra can be explained by the quantum

confinement effect, which is a result of the confinement of electrons and holes within a limited volume of the nanocrystal. The phenomenon under consideration leads to the quantization of energy levels of both electrons and holes thereby causing alterations in the optical and electronic characteristics of the nanocrystals that are dependent on their size. The development of synthetic methods for quantum dots was deemed a significant contribution. The synthesis of colloidal quantum dots through solution-phase techniques was pioneered by Louis E. Brus and R. Murray. The techniques employed in this study entailed the introduction of precursor substances into a coordinating solvent, succeeded by the incorporation of a capping agent to regulate the dimensions and morphology of the quantum dots. The quantum dots that were produced exhibited a limited range of sizes, a high quantum yield, and robust luminescence, rendering them appealing for utilisation in a diverse array of applications. In the initial stages of research, quantum dots were subjected to diverse methodologies to determine their properties [53]. The optical properties of quantum dots were investigated using absorption and emission spectroscopy, while their structural properties were examined through X-ray diffraction and transmission electron microscopy. The researchers additionally examined the impacts of dimensions, morphology, and constitution on the characteristics of quantum dots. Early researchers have demonstrated the potential applications of quantum dots in various fields. The distinct optical and electronic characteristics of quantum dots were demonstrated by Paul Alivisatos and Moungi Bawendi, which could be utilised for various purposes including optoelectronics, sensing, and biolabeling [54]. The research conducted by the authors established the fundamental principles for the emergence of quantum dots as a distinct area of study, and facilitated their integration into a diverse array of contemporary applications. In brief the finds can be uttered like these:

- The quantum confinement effect was initially observed by early researchers. This phenomenon causes the energy levels of electrons and holes in a nanocrystal to become quantized. Consequently, the optical and electronic properties of the nanocrystal undergo size-dependent changes [55].
- The researchers additionally devised synthetic procedures for quantum dots through solution-phase methodologies, yielding nanocrystals characterised by a limited size range, elevated quantum efficiency, and robust luminescence.
- Quantum dots were characterised by early researchers through a range of techniques such as absorption and emission spectroscopy, X-ray diffraction, and transmission electron microscopy.
- The researchers conducted an investigation into the impact of quantum dot size, shape, and composition on their respective properties.
- The initial researchers exhibited the potential uses of quantum dots in domains such as optoelectronics, sensing, and biolabeling, thereby establishing the groundwork for their current utilisation in a diverse array of applications.

3 Advancements in Quantum Dot Synthesis

Various techniques have been devised for the production of quantum dots, each possessing unique merits and drawbacks. Several frequently employed techniques include:

1. The colloidal synthesis method is a commonly employed technique for the production of quantum dots in a liquid medium. The present technique involves the dissolution of precursors in a suitable solvent, subsequent to which a reducing agent and stabilising agent are introduced. Quantum dots are generated via a process of nucleation and growth.
2. The sol-gel synthesis technique entails the chemical reactions of hydrolysis and condensation of metal alkoxides in a solution, resulting in the formation of a substance with a gel-like consistency. Subsequently, the gel is subjected to a drying process and subsequently exposed to heat in order to generate the quantum dots.
3. The process of chemical vapour deposition entails the application of a slender layer of a precursor substance onto a substrate, succeeded by the reaction of the precursor with a reactant in the gas phase, resulting in the formation of quantum dots.
4. The electrochemical synthesis technique entails the deposition of metal ions onto a substrate while a reducing agent is present, resulting in the formation of quantum dots.
5. The process of plasma synthesis entails the utilisation of a plasma discharge to produce exceedingly reactive species that can interact with precursor gases, resulting in the formation of quantum dots.
6. The process of laser ablation entails utilising a laser beam to ablate a solid target, thereby producing plasma that can interact with a gas-phase reactant to create quantum dots.

Each of the aforementioned techniques possesses unique benefits and drawbacks with respect to regulating the dimensions, morphology, and chemical makeup of the quantum dots, as well as their potential for expansion and consistency in results. The selection of the appropriate technique is contingent upon the particular usage scenario and the intended characteristics of the quantum dots.

4 Discussion of the Role of Surface Chemistry in Quantum Dot Synthesis

Quantum dots are semiconductor materials at the nanoscale level that possess distinctive electronic and optical characteristics, rendering them exceedingly appealing for utilisation in various domains, including but not limited to biological imaging, optoelectronics, and energy. The process of synthesising quantum dots is intricate and

demands meticulous regulation of the nanocrystals' size, shape, and composition. The significance of surface chemistry in quantum dot synthesis cannot be overstated, as it has a profound impact on the growth kinetics, stability, and properties of the nanocrystals [56]. The manipulation of the surface chemistry of quantum dots can be achieved through the incorporation of ligands or surface coatings during the process of synthesis. The ligands exhibit an interaction with the surface of the quantum dots, thereby influencing their size, shape, and properties. Ligands serve multiple pivotal functions in the process of quantum dot fabrication [57]. Primarily, they function as capping agents that impede the agglomeration of quantum dots, which is a pivotal element in regulating the dimensions of the nanocrystals. The presence of ligands induces steric hindrance among the quantum dots, thereby constraining their capacity to approach one another and coalesce into aggregates. The growth kinetics of nanocrystals can be influenced by ligands, which have the ability to modulate the surface energy of quantum dots. The quantum dot's surface energy plays a crucial role in determining its propensity to accept atoms or molecules during the growth process, thereby influencing its size and morphology [58]. The modulation of surface energy through the use of ligands can effectively decelerate the rate of nanocrystal growth, ultimately resulting in the production of smaller and more homogenous particles. In addition, it should be noted that ligands have the potential to modify the surface charge of quantum dots, thereby affecting their stability and interactions with chemical or biological surroundings [4, 59–61]. The determination of nanoparticle stability in a solution is reliant on the crucial factor of surface charge. The manipulation of surface charge by ligands can establish a protective enclosure around quantum dots, thereby impeding their aggregation or interaction with other molecules present in the solution. The selection of ligands employed in the process of synthesis plays a crucial role in determining the surface chemistry of quantum dots [62]. This, in turn, has a significant impact on their properties and potential applications. Hydrophilic ligands, such as carboxylic acids or amines, can generate a water-soluble surface, which facilitates the utilisation of quantum dots in biological imaging and sensing applications. In contrast, ligands that exhibit hydrophobic properties have the ability to augment the stability of quantum dots in solvents that lack polarity, thereby conferring utility in optoelectronic domains.

Over the years, there have been notable developments in the production of quantum dots. Presented below is a concise summary of some of the principal progressions.

The initial methods of synthesising quantum dots involved colloidal synthesis and were accomplished in the early 1990s. Subsequently, additional methodologies, including sol-gel synthesis and chemical vapour deposition, were also established. The technique of size-tunable synthesis was developed by researchers in the late 1990s, which enabled accurate manipulation of the size of quantum dots. The aforementioned outcome was attained via alterations in the reaction parameters, including but not limited to adjustments in temperature, duration, and concentration of the precursor substances [63]. The development of high-quality synthesis methods in the early 2000s resulted in significant progress in the production of quantum dots with superior optical and electronic properties, as reported by researchers. The

aforementioned outcome was attained through the utilisation of materials with high levels of purity and the optimisation of synthesis conditions to reduce the occurrence of defects [64]. The development of quantum dots has garnered increasing attention in recent times, with a focus on utilising novel materials like perovskites and metal-organic frameworks. The aforementioned materials possess distinctive benefits, including elevated quantum yields and adjustable bandgaps [65]. The escalating demand for quantum dots across diverse applications has prompted a concentration on the advancement of large-scale synthesis techniques. Recent developments in this field encompass the implementation of continuous flow synthesis and microwave-assisted synthesis techniques.

5 Properties of Quantum Dots

The optical properties of quantum dots are dependent on their size, shape, and composition, which give rise to their distinctive characteristics. The quantization of energy levels arises due to the confinement of electrons and holes within a small volume in quantum dots, resulting in size-dependent properties. As the dimensions of the quantum dot decrease, the energy levels exhibit greater discreteness, leading to an increase in the bandgap and a corresponding shift in the absorption and emission spectra towards higher energies [66]. The phenomenon being referred to is commonly recognised as the quantum confinement effect. The surface area-to-volume ratio of a quantum dot is influenced by its size, which in turn affects its reactivity and stability, as well as its bandgap.

The properties of quantum dots can be significantly influenced by their shape [67–70]. Quantum dots that are anisotropic in nature, such as nanorods or nanowires, demonstrate absorption and emission spectra that are dependent on polarisation. This is attributed to the alignment of the dipoles along the longitudinal axis of the particle [71]. The electronic structure and optical properties of a quantum dot can be altered by its shape, resulting in modifications to its energy levels. Quantum dots possessing faceted geometries may manifest distinct surface terminations, thereby influencing their surface chemistry and stability.

The properties of a quantum dot can be significantly influenced by its composition, particularly its chemical makeup. The determination of the bandgap of a quantum dot is contingent upon the disparity in energy levels between the conduction and valence bands, a factor that is subject to the quantum dot's size and composition [72]. Cadmium-based quantum dots are known to demonstrate a greater bandgap in comparison to lead-based quantum dots of equivalent size. The chemical composition of a quantum dot can have a significant impact on its surface chemistry, thereby influencing its stability and reactivity.

The optical characteristics of quantum dots have garnered significant attention owing to their potential utility in diverse domains [73]. Quantum dots possess a narrow emission spectra and high quantum yields, rendering them suitable for deployment as fluorescent probes. Furthermore, the tunability of quantum dots' emission

spectra can be achieved through alterations in their size, shape, and composition, rendering them remarkably versatile [74]. Quantum dots possess the capability to be deliberately designed to emit at particular wavelengths within the visible or near-infrared spectrum, thereby rendering them advantageous for employment in imaging and sensing applications.

To summarise, the characteristics of quantum dots are significantly influenced by their dimensions, morphology, and chemical makeup. Comprehending the aforementioned characteristics holds paramount significance in customising quantum dots for particular uses in domains such as optoelectronics, biomedicine, and energy transformation. Furthermore, current investigations within the discipline are concentrated on enhancing the stability and quantum yield of quantum dots, while also delving into novel applications for these distinctive nanomaterials.

6 Overview of the Application of Quantum Dots in Various Fields

Semiconductor nanoparticles known as quantum dots possess distinctive electronic and optical characteristics that render them highly advantageous instruments across a range of disciplines. Nanocrystals are commonly composed of materials like cadmium selenide, cadmium telluride, and indium arsenide, and exhibit a size distribution ranging from a few to several hundred nanometers. The field of optoelectronics has witnessed a significant utilisation of quantum dots. Quantum dots possess distinctive optical characteristics owing to their high surface-to-volume ratio, which is attributed to their diminutive size. Particularly, they possess the ability to produce light at exact wavelengths, rendering them suitable for implementation in superior quality screens, light-emitting diodes, and photovoltaic cells. The capacity of emitting light in various hues, coupled with their elevated luminosity, renders them advantageous in the context of illuminative purposes. Quantum dots have the potential to serve as a labelling tool for biological molecules and cells in the field of biological imaging, thereby facilitating their visualisation and monitoring. Compared to conventional organic dyes, they present various benefits, including enhanced luminosity, improved resistance to photodegradation, and narrower emission spectra. Moreover, the diminutive dimensions of quantum dots render them capable of infiltrating tissues to a significant extent, thereby rendering them well-suited for deployment in the realm of in vivo imaging. The utilisation of quantum dots in the domain of energy production is currently under investigation. Quantum dots have the ability to absorb a wider spectrum of light wavelengths in comparison to conventional solar cells, thereby enhancing their capacity to transform solar energy into electrical energy. Furthermore, quantum dots exhibit promising prospects for utilisation in energy storage systems, including batteries and supercapacitors. Quantum dots are currently under investigation in the realm of information technology due to their potential to serve as qubits, which are fundamental units in the construction of quantum computers.

The utilisation of quantum dots is applicable in the advancement of memory and data storage devices that possess high-speed and high-capacity capabilities. Quantum dots exhibit potential for utilisation in sensing and detection applications. These devices have the capability to function as sensors for detecting alterations in environmental factors such as temperature, pressure, and others. Furthermore, they have the capability to detect and distinguish diverse categories of molecules such as biomolecules, contaminants, and explosives. Quantum dots exhibit distinctive electronic and optical characteristics that render them highly favourable for diverse applications spanning multiple domains. Anticipated progress in research endeavours is poised to yield noteworthy breakthroughs in the utilisation and creation of these nanoparticles across domains encompassing energy, healthcare, and information technology.

7 Quantum Dot Technology

The distinct characteristics exhibited by quantum dots have resulted in their utilisation across a diverse array of technological domains (Fig. 1). The principal implementations of quantum dots are as follows.

Quantum dots have found extensive application in the realm of display technology, particularly in LED backlit displays. The utilisation of these elements results in the

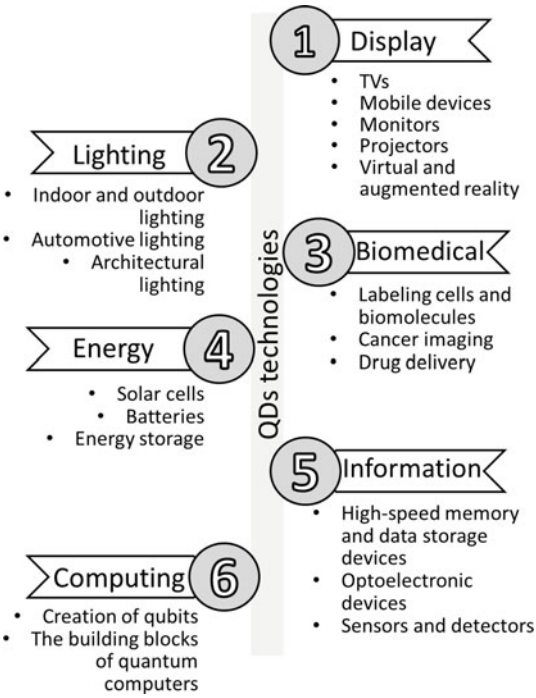

Fig. 1 Various types of applications of QDs nanoparticles

generation of hues that are more pronounced, accompanied by an elevated degree of luminosity and differentiation [75]. Conventional LED displays typically employ white LEDs in conjunction with colour filters to generate diverse hues; however, this approach may lead to energy dissipation and a reduced range of colours. In contrast, displays utilising quantum dots employ blue light emitting diodes (LEDs) in conjunction with quantum dots that generate red and green light upon being stimulated by the blue light source. The outcome of this is an expanded range of colours and a display that consumes less energy.

Quantum dots exhibit distinctive optical characteristics that render them valuable for medical imaging purposes. Fluorescent tags can be utilised for the purpose of identifying and tracking particular cells or molecules within the human body. Quantum dots possess the ability to be tailored to emit light at precise wavelengths, rendering them highly suitable for imaging purposes that necessitate superior resolution and sensitivity [76]. Furthermore, they are currently under development as contrast agents to facilitate more accurate imaging of neoplasms and other anomalous tissues.

The utilisation of quantum dots is being explored as a means of enhancing the efficacy of solar cells through the capture of a wider range of light wavelengths, which can subsequently be converted into electrical energy [77]. Solar cells conventionally comprise crystalline silicon and possess a limited capacity to absorb light wavelengths. The incorporation of quantum dots into the solar cell enables the capture of a wider range of light wavelengths, thereby facilitating the conversion of light energy into electrical energy [49]. The utilisation of quantum dots in conjunction with other materials has the potential to yield multi-junction solar cells that exhibit superior levels of efficiency.

Quantum dots have the potential to serve as qubits within quantum computing frameworks. Qubits serve as the fundamental units of quantum computers and possess the ability to exist in numerous states concurrently, thereby facilitating expedited processing as compared to conventional computers. Quantum dots exhibit extended coherence times and are amenable to facile manipulation, rendering them a propitious substrate for constructing quantum computers on a macroscopic scale.

Quantum dots are currently being utilised in the development of energy-efficient lighting solutions that offer superior quality [16, 49, 69]. Light-emitting diodes (LEDs) have the potential to serve as a substitute for conventional fluorescent or incandescent bulbs, as they are capable of emitting light with a broader spectrum of colours and a greater luminosity. Quantum dot lighting possesses the added benefit of enhanced energy efficiency, thereby resulting in considerable cost reductions in the long run.

Quantum dots are currently under development for various biotechnology purposes such as drug delivery, biomolecule detection, and disease diagnosis. Nanoparticles possess the capability to selectively target particular cells or molecules, thereby rendering them a potent instrument in the realm of nanomedicine. Quantum dots possess the capability of being tailored to selectively bind with particular proteins or nucleic acids, thereby enabling their application in the detection of biomolecules or diagnosis of diseases.

In general, quantum dots exhibit a broad spectrum of potential applications across diverse domains, and continued scientific inquiry and innovation is anticipated to yield further compelling utilities in the times ahead. Quantum dots possess distinctive characteristics that render them highly adaptable and multifaceted, thereby positioning them as a technology with considerable potential for diverse applications.

The field of optoelectronics has shown interest in utilising quantum dots due to their distinctive optical characteristics. Quantum dots exhibit a quantized energy spectrum owing to their diminutive size, resulting in the emission of light at distinct wavelengths. The aforementioned characteristic renders them highly suitable for utilisation in light-emitting diodes and laser devices, wherein meticulous regulation of the emanated wavelengths holds paramount significance. Quantum dots exhibit a broad absorption spectrum, enabling them to capture light across a diverse range of wavelengths. The aforementioned characteristic holds practical significance in down-conversion scenarios, wherein photons with high energy levels are transformed into photons with lower energy levels that can be assimilated by conventional semiconductor materials, such as silicon.

Furthermore, quantum dot light-emitting diodes (QLEDs) possess the capability to offer superior visual displays featuring an extensive range of colours. Quantum dots have the capability to emit light across a diverse range of colours, encompassing those within the visible spectrum. Quantum dot light-emitting diodes (LEDs) exhibit high efficiency and are capable of generating vivid and intense hues, rendering them a compelling substitute for conventional LEDs in lighting and display contexts.

Quantum dots have been identified as a potential material for advanced solar cells owing to their distinctive characteristics. Quantum dots possess a significant benefit in their ability to effectively absorb light across a wide spectrum of wavelengths. This implies that the light-capturing capacity of conventional semiconductor materials, such as silicon, is restricted to a limited range of wavelengths, whereas the aforementioned materials can capture a broader spectrum of light. Furthermore, quantum dots possess the capability to function as down-converting layers. This feature facilitates the enhancement of the light-capturing capacity of conventional silicon solar cells by transforming high-energy photons into lower-energy photons that can be assimilated by silicon.

Quantum dots possess the capability to enhance the efficacy of solar cells by enabling them to apprehend light in the infrared segment of the spectrum, which is beyond the reach of silicon-based solar cells. The reason for this is that quantum dots can be customised to possess a band gap that can be adjusted to the targeted wavelength spectrum. The integration of quantum dots into photovoltaic cells is being investigated by scholars as a means of enhancing their efficacy and diminishing the expense of solar power.

The utilisation of quantum dots as qubits, which are the fundamental units of quantum computers, is a viable option in the field of quantum computing. The utilisation of the spin of an electron in a quantum dot as a qubit and the exploitation of the quantum properties of these systems for the purpose of executing quantum computations can be achieved. The scalability of quantum dots and their compatibility with current semiconductor manufacturing processes make them a promising

technology. This implies that they possess the capability of being manufactured in significant quantities and incorporated into pre-existing electronic apparatus.

Nevertheless, there are still obstacles to be overcome with regards to preserving the consistency of the qubits for extended durations. The reason for the susceptibility of qubits to decoherence is attributed to the high sensitivity of quantum dots to their surrounding environment, whereby any form of noise or fluctuations can result in the loss of coherence. Scholars are currently engaged in the development of methodologies aimed at regulating the ambient conditions surrounding quantum dots, with the objective of mitigating the deleterious effects of noise on their operational efficacy.

The distinctive optical characteristics of quantum dots render them a desirable option for application in the fields of biotechnology and medical imaging. Fluorescent probes have the potential to serve as tracking agents for cellular or molecular movements within biological systems. Quantum dots exhibit exceptional stability and emit bright and persistent light, rendering them a highly suitable option for various imaging applications. Moreover, quantum dots have the potential to serve as contrast agents in medical imaging, offering superior resolution and heightened sensitivity in comparison to conventional imaging techniques.

Quantum dots possess potential for utilisation in drug delivery and therapeutic applications. Due to their diminutive dimensions, nanoparticles possess the ability to infiltrate cells and tissues. Additionally, their surface can be modified with targeting molecules, thereby enabling the transportation of therapeutic agents or drugs to precise cells or tissues.

Quantum dots exhibit potential applications in diverse fields including catalysis, sensing, and data storage performance in various chemical reactions. The electronic and optical properties of nanoparticles, which are dependent on their size, have been found to have a significant impact on their catalytic activity. As a result, they have been employed as catalysts in a variety of chemical reactions.

8 Commercialization of Quantum Dots

The inception of quantum dots' commercialization dates back to the latter part of the 1990s. Subsequently, there has been a notable surge in their manufacturing and utilisation. Quantum dots are extensively utilised in display technology as a primary application, facilitating the creation of energy-efficient and high-quality displays. Quantum dots have been employed in medical imaging due to their ability to selectively bind to particular tissues and produce more precise images compared to conventional imaging methods. Quantum dots have recently gained traction in the realm of quantum computing as a potential application, serving as fundamental units or qubits for quantum computers. The potential application of quantum dots in solar cells is currently under investigation, with the aim of enhancing the efficiency of the cells and decreasing the expenses associated with solar energy production. The commercialization of quantum dots has encountered various obstacles, such as the exorbitant

expenses associated with their manufacturing and apprehensions regarding their plausible toxicity. Notwithstanding the challenges, scholars and enterprises are endeavouring to surmount them by devising novel production techniques and investigating measures to enhance the safety of quantum dots for human utilisation.

The process of commercialising quantum dots has been in progress for a number of years, and it is anticipated that the quantum dots market will experience substantial growth in the near future. Several prominent corporations are engaged in the process of commercialising quantum dots.

1. Nanosys is a prominent enterprise that specialises in the advancement and monetization of quantum dots. The corporation provides a variety of display technology products, such as quantum dot films, LED backlights, and colour conversion layers.
2. QD Vision is a prominent enterprise that specialises in the advancement and dissemination of quantum dots. The corporation provides quantum dot merchandise that caters to display technology, lighting, and biomedical applications.
3. Samsung is a significant participant in the market for quantum dot displays. The QLED (Quantum Dot LED) technology employed by the company is utilised in its premium-grade televisions and monitors.
4. LG is a significant participant in the quantum dot display industry. The NanoCell TVs manufactured by the company utilise quantum dot technology to augment colour precision and luminosity.
5. Quantum Materials Corp is a corporation that specialises in the research, development, and manufacturing of quantum dots for a wide range of applications, including but not limited to display, lighting, solar cells, and biomedical imaging.
6. Crystalplex Corporation is a specialised enterprise that focuses on the advancement of quantum dots for the purpose of biomedical imaging and diagnostics.
7. NN-Labs is a corporation that specialises in the production of top-notch quantum dots for various purposes such as LED illumination, display technology, and biomedical imaging.
8. Ocean NanoTech is a commercial enterprise that specialises in the manufacture and distribution of quantum dots, which are utilised in various fields such as imaging, diagnostics, and sensing.
9. Quantum Solutions is a corporation that specialises in the production of top-tier quantum dots utilised in various applications, including but not limited to solar cells, LED lighting, and display technology.

Other leading companies are also in market which is depicted in Table 1.

Table 1 Different companies those are working in quantum dots and their focus on the market

Company	Industry	Focus
Max Planck Institute	Academic/research	Quantum computing, sensing
Nanosys	Materials science	Quantum dot films for display technology
QD Vision	Materials science	Quantum dots for display technology and lighting
Quantum Materials Corp	Materials science	Quantum dots for display technology, solar cells, and biomedical imaging
NN-Labs	Materials science	Quantum dots for led lighting, display technology, and biomedical imaging
Ocean NanoTech	Materials science	Quantum dots for biomedical imaging, diagnostics, and sensing
Crystalplex Corporation	Biotechnology	Quantum dots for biomedical imaging and diagnostics

9 Challenges and Opportunities in the Commercialization of Quantum Dots

Quantum dots exhibit several characteristics that render them a highly favourable material for a diverse array of applications, such as optoelectronics, biotechnology, and energy harvesting. Notwithstanding, there exist a number of obstacles and prospects that necessitate resolution in order to effectively bring quantum dots to market.

Challenges:

The commercialization potential of quantum dots is limited due to the relatively high production cost of producing high-quality ones. The elevated expense can be attributed to the intricate process of synthesis, utilisation of costly initial components, and the requirement for specialised equipment. The presence of heavy metals, such as cadmium, in certain quantum dots has raised concerns regarding their potential toxicity during production and use. The development of non-toxic and biocompatible quantum dots is imperative for their effective utilisation in biomedical applications. The long-term stability of quantum dots is limited due to their susceptibility to degradation, which ultimately impacts their performance. The cause of this instability can be attributed to various factors, including exposure to light, oxygen, and temperature fluctuations. This presents a notable obstacle that must be overcome in order to facilitate the commercialization of these products.

Opportunities:

Quantum dots exhibit distinctive optical characteristics that render them advantageous for various optoelectronic applications, including but not limited to display technology, lighting, and solar cells. Quantum dot displays exhibit superior colour gamut, colour accuracy, and energy efficiency in comparison to conventional LCD

displays. The field of biotechnology has shown great potential in utilising quantum dots as effective instruments for biomedical imaging and drug delivery. Quantum dots possess high luminosity and stability, and their emission characteristics can be adjusted to precise wavelengths, rendering them valuable for visualising particular biological structures and functions. Quantum dots possess the capability to absorb light across a broad spectrum of wavelengths, rendering them a viable option for solar energy harvesting. These can be utilised to augment the efficacy of photovoltaic cells, and to fabricate novel substances for energy retention.

To summarise, the commercialization of quantum dots encounters various obstacles such as high production expenses, apprehensions regarding toxicity, and durability. Quantum dots have emerged as a promising material for a diverse array of applications, including optoelectronics, biotechnology, and energy harvesting, presenting significant opportunities in these fields. The realisation of the complete potential of quantum dots in the future will necessitate the resolution of challenges and the development of novel applications.

10 Future of Quantum Dots

The particular optical, electrical, and physical characteristics of quantum dots render them a propitious domain of investigation. Quantum dots are utilised in optoelectronics for various purposes, including but not limited to display technology, lighting, and solar cells. Ongoing research endeavours are focused on enhancing the performance and efficiency of quantum dot displays, exploring novel lighting technologies, and optimising the efficacy of solar cells. Quantum dots exhibit promising potential in the realm of biomedical applications, specifically in the areas of drug delivery and imaging. Scholars are currently exploring methods to enhance the biocompatibility of quantum dots and mitigate their toxicity. Additionally, they are devising novel imaging techniques and targeting strategies for drug administration. Quantum dots exhibit promise in the realm of quantum computing owing to their capacity to confine and manoeuvre individual electrons. Scholars are currently investigating potential applications of quantum dots in the development of qubits, which serve as the fundamental building blocks of quantum information, with the aim of enhancing the efficacy of quantum computing platforms. Quantum dots have potential applications in energy storage, including but not limited to batteries and capacitors. Ongoing research endeavours are focused on the development of novel materials and devices for the purpose of energy storage, utilising quantum dots. Quantum dots exhibit potential applications in the realm of environmental monitoring and remediation, including the detection of pollutants and the augmentation of water purification processes. Ongoing research endeavours are focused on the development of novel sensing and treatment technologies utilising quantum dots. In recent decades, scholars have achieved notable advancements in comprehending the conduct of quantum dots and devising novel techniques for their amalgamation and integration into apparatus. The following discourse outlines potential advancements and future trajectories in

the realm of quantum dot research and its practical applications. Quantum dot sensors have been employed for the detection of minute variations in temperature, pressure, and magnetic fields. Currently, scholars are investigating the potential of quantum dots as biosensors for the identification of biomolecules, including proteins and DNA. The potential applications of these biosensors encompass medical diagnostics and drug discovery. The utilisation of quantum dots in solar cells has been shown to increase their efficacy by augmenting light absorption and subsequent conversion into electrical energy. Scientists are currently engaged in enhancing the reliability and expandability of solar cells that are based on quantum dots, with the aim of rendering them feasible for commercial purposes. The utilisation of quantum dots as qubits in quantum computing is a promising avenue for exploration. Quantum dots are being investigated by researchers as a potential foundation for constructing quantum computers that exhibit greater stability and scalability in comparison to alternative qubit configurations. Quantum dot-based light-emitting diodes (LEDs) have been developed as a promising solution for efficient and adjustable lighting and display applications. Scholars are currently engaged in enhancing the effectiveness and chromatic accuracy of light-emitting diodes that utilise quantum dots, with the aim of rendering them comparable to conventional LEDs. Quantum dots have the potential to serve as memory devices in electronic systems. Quantum dots are being investigated by scholars as a potential non-volatile memory storage alternative that can be downsized to dimensions smaller than those of existing memory technologies. To attain these advancements and fully exploit the capabilities of quantum dots, it is imperative for researchers to enhance their comprehension of the fundamental physics of quantum dots and devise novel techniques for their synthesis and integration into devices. Furthermore, the progression of fabrication and manufacturing methodologies will play a crucial role in the expansion of quantum dot-centered devices for commercial purposes.

11 Conclusion

The potential application of quantum dots in solid-state lighting, specifically in LED lights, is a noteworthy impact. The utilisation of quantum dots as colour conversion agents enables the generation of white light that exhibits superior energy efficiency and colour rendering characteristics in comparison to conventional lighting sources.

Quantum dots are currently being investigated in the realm of biology and medicine due to their potential applications in targeted drug delivery and gene therapy. Biological molecules can be applied as a coating to enable targeted delivery of drugs or genes to affected cells or tissues, thereby minimising the risk of unintended impacts. Quantum dots possess the capability to transform the domain of quantum cryptography by facilitating the creation and transmission of secure quantum keys for data encryption. Similar to other nascent technologies, quantum dots present certain potential hazards, including apprehensions regarding toxicity and ecological consequences. Scholars are currently investigating methods to tackle these issues

and guarantee the secure and ethical advancement of quantum dot technology. To conclude, quantum dots represent an intriguing and auspicious realm of investigation that harbours the possibility of transforming diverse domains of science and technology. Due to their distinctive characteristics and adaptability, they are deemed indispensable instruments for scientists and professionals who are engaged in the advancement of novel applications and technologies.

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Synthesis Strategies of Doped QDs

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Abstract The implementation of synthesis strategies for doped QDs (QDs) is of paramount importance in the customization of their electronic and optical characteristics, thereby facilitating their utilisation in a diverse array of fields such as optoelectronics, biomedicine, energy, sensing, and others. This abstract presents a succinct summary of the synthesis methodologies utilised in the fabrication of doped QDs (QDs). The aforementioned tactics encompass codoping, post-synthetic doping, ion exchange, alloying, and core-shell architectures. The process of codoping entails the concurrent introduction of multiple dopants during the synthesis of QDs, thereby enabling accurate regulation of their concentration and amalgamation. The process

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of post-synthetic doping involves the introduction of dopants onto pre-synthesized QDs (QDs) through surface modification or ligand exchange procedures. The process of ion exchange involves the substitution of dopant ions for the original cations or anions present in the lattice of QDs. The process of alloying involves the combination of distinct semiconductor materials, with the inclusion of dopants during the alloying procedure. Core-shell architectures facilitate the encapsulation of doped QDs (QDs) within a safeguarding shell, thereby providing the ability to regulate dopant diffusion and associated characteristics. The utilisation of synthesis strategies offers a degree of adaptability in customising the characteristics of doped QDs to suit particular applications. The objective of future investigations in this domain is to devise novel doping methodologies and refine current techniques with the aim of augmenting the efficacy and broadening the scope of applications of doped QDs.

Keywords Doped QDs · Codoping · Encapsulation · Biomedicine · Optoelectronics

1 Introduction

QDs are semiconductor particles at the nanoscale that display distinctive optical and electronic characteristics as a result of quantum confinement phenomena [1, 2]. In recent times, there has been a notable surge in interest towards them owing to their prospective utilisation in diverse domains, including optoelectronics, biomedicine, and renewable energy [3–6]. The extent to which their properties can be adjusted is restricted by their dimensions and constituents. The utilisation of impurities to dope QDs has emerged as a promising strategy to overcome their limitations and improve their performance. The process of doping QDs entails the incorporation of dopants or impurities into the crystal lattice of the semiconductor material [1]. The alteration of the electronic structure and bandgap of QDs through this process can result in modifications of their optical and electronic characteristics. Doping has the potential to enhance the stability, biocompatibility, and solubility of QDs, thereby expanding their applicability across various domains [7]. Numerous methodologies have been devised to fabricate doped QDs with regulated composition and characteristics. The aforementioned strategies can be categorised into two distinct groups: (1) doping after synthesis and (2) co-doping during the synthesis process. The process of post-synthesis doping pertains to the incorporation of dopants into QDs that have already undergone synthesis. The aforementioned methodology facilitates the alteration of characteristics of extant QDs while preserving their dimensions and morphology. Ion implantation is a frequently utilised technique for post-synthesis doping [8]. The process of introducing dopants into QDs involves ion bombardment. The ion implantation methodology is characterised by a high degree of precision and enables the incorporation of a diverse array of dopants. Nevertheless, it has the potential to impair the crystal structure of the QDs and diminish their efficacy in emitting light.

Molecular doping represents an alternative strategy for post-synthesis doping [9]. The present technique involves the introduction of dopants into the QDs through either surface adsorption or lattice diffusion. The utilisation of this particular methodology is comparatively less deleterious to the crystal structure of QDs in contrast to ion implantation, while also having the capability to incorporate a more extensive spectrum of dopants. Nevertheless, the process of molecular doping may result in the formation of quantum dot aggregates, thereby diminishing their stability. The process of co-doping in the synthesis of QDs refers to the concurrent integration of dopants into the synthesis procedure. The aforementioned methodology facilitates meticulous regulation of the QDs' composition and characteristics, while concurrently mitigating the incidence of flaws and impairment to the crystal lattice. The hot-injection method is a frequently employed technique for co-doping. The present methodology involves the injection of a blend of precursor substances and dopants into a heated solution to produce QDs with regulated composition and characteristics. The hot-injection technique is a remarkably effective approach that enables the production of doped QDs of exceptional quality while providing accurate regulation over their characteristics [10]. The one-pot synthesis method represents an alternative approach to co-doping. The present methodology involves the introduction of a blend of precursor substances, dopants, and ligands into a solitary reaction container to effectuate the production of doped QDs in a unified step [11]. The aforementioned methodology is characterised by its straightforwardness and effectiveness, as well as its capacity to generate substantial volumes of doped QDs. The intricate reaction environment may pose challenges in regulating the characteristics of the QDs produced. Various dopants have been employed to alter the characteristics of QDs, encompassing metals, metal oxides, rare-earth elements, and organic molecules. The incorporation of dopants has the potential to modify the optical and electronic characteristics of QDs, including their quantum yield, emission wavelength, and bandgap [12–14]. The selection of a dopant is contingent upon the intended characteristics of the QDs and their particular usage. To sum up, the introduction of impurities into QDs through doping has surfaced as a propitious method for altering their characteristics and amplifying their efficacy. Various techniques have been devised to fabricate doped QDs with regulated composition and characteristics, such as doping after synthesis and co-doping during synthesis [15–20].

2 Different Doping Strategies of QDs

Doping is an effective tool for modifying the characteristics of QDs. Doping of impurities into QDs can be broadly categorised as either extrinsic or intrinsic doping.

2.1 Extrinsic Doping Strategies

The traditional technique of doping, which involves the introduction of impurity atoms, proved to be ineffective in several initial instances due to the tendency of the impurities to be expelled from the small crystalline cores. Additionally, the thermal ionization of impurities, which generates free carriers, was impeded by strong confinement. A potential avenue for addressing these limitations involves the extrinsic introduction of dopant ions. The study conducted by Guyot-Sionnest et al. [21, 22] explored into the examination of colloidal QDs composed of different materials, namely CdSe, CdS, and ZnO. The results of the study demonstrated that these materials can undergo n-type reduction through the use of Na, K, biphenyl radical anions, or electrochemical doping, wherein electrons occupy the quantum confined states of the conduction band. The present study showcases the impact of sodium biphenyl on the infrared and visible absorption spectra of CdSe nanocrystals. The results revealed a noticeable reduction in the intensity and broadening of the first and second excitonic peaks, indicating bleaching. The manifestation of the 1Se–1Pe infrared absorption peak at 0.3 eV provides evidence for the n-type nature of the nanocrystals. Additional prospects encompass the investigation of the electrical conductivity of n-type QDs films, attributed to their increased inter-QD electron transfer, which may yield photovoltaic or electronic implementations [21, 23]. Another technique for extrinsic doping involves the utilization of a Bronsted base, such as hydrazine, to modify the surface of the quantum dot (QD). This process replaces the bulky oleic acid groups, resulting in a reduction of intermolecular spacing. Additionally, the dangling bonds on the surface are passivated by the lone pair of electrons on the base, which is comparable to primary amines. The improvement of conductivity in QD solids is facilitated by this process, thereby enhancing the efficacy of QDs in diverse applications such as field effect transistors. The conductance of PbSe nanocrystal solids was observed to escalate by ten orders of magnitude subsequent to hydrazine treatment. The technique has been demonstrated to have a broad range of applications on various materials; however, its widespread applicability is constrained by the instability of the ligands involved [24]. In recent times, hydrazine molecules have been substituted with more stable and environmentally friendly molecules, thereby showcasing the extended practicality of this approach [25].

2.2 Intrinsic Doping

Extrinsic doping is not a very feasible choice for imparting functionalities such as magnetic, optical, or optoelectronic functions, despite the modest results that have been achieved with this technique. As a result, it is essential to find procedures that are capable of producing effective intrinsic doping of dopant ions [26]. While doping has been widely conducted and has yielded high-quality QDs (QDs) in certain instances,

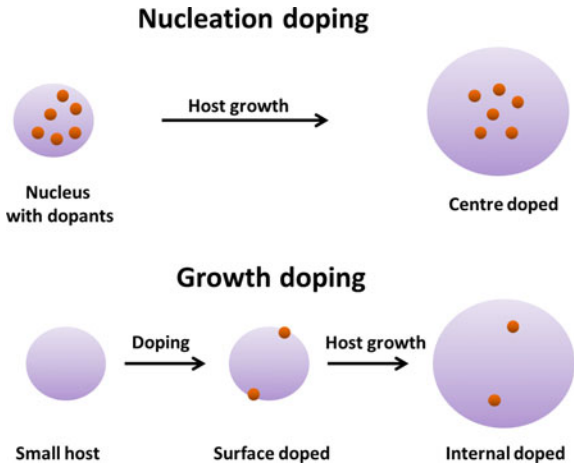

Fig. 1 Graphical representation of the proposed radial positions of the dopant in nucleation and growth doping

Pradhan et al. were the first to propose mechanistically significant techniques for doping Mn and Cu in ZnSe-based systems [26]. The authors proposed techniques to dissociate the dopant and host precursors to account for their distinct reactivities. These techniques involve nucleation or growth doping to achieve efficient doping of the QDs, as illustrated in Fig. 1.

In the context of nucleation doping, the initial step involves the formation of a small dopant compound. In the context of nucleation doping, a small dopant compound was initially generated. Subsequently, the aforementioned core nuclei were coated with a host shell, leading to the formation of either graded alloys or sharp interfaces, dependent upon the prevailing conditions. In the context of growth doping, the authors presented evidence of the emergence of small host QDs, succeeded by the adsorption of the dopant ion on the surface at a reduced temperature, and the introduction of the dopant metal precursor. In order to encapsulate the dopant ions, isocrystalline or heterocrystalline shells were formed, which demonstrates the extensive applicability of this method [3, 27]. As shown in Fig. 2, effective doping and decoupling from nucleation and growth are demonstrated by the rise in the photoluminescence (PL) intensity of the dopant emission and the reduction in the PL intensity of the host (top, left). This prototype demonstration of the proposed mechanism was carried out for Cu or Mn-doped ZnSe QDs. The PL undergoes a redshift as a consequence of the regrowth of ZnSe on the surface of the doped QDs (top, right). Because it took 100 min to finish, the procedure described above would have been tough to control if it hadn't been for the decoupling technique. On the other hand, nucleation doping was accomplished for Mn-doped ZnSe by utilizing a variety of dopant precursors. Due to the fact that Mn^{2+} is a harder Lewis acid than Zn^{2+}, it is anticipated that the Mn^{2+} precursor will exhibit lower levels of reactivity when combined with the same ligand. When manganese stearate was utilised as the nucleation precursor, zinc acetate was preferred over zinc stearate for the production

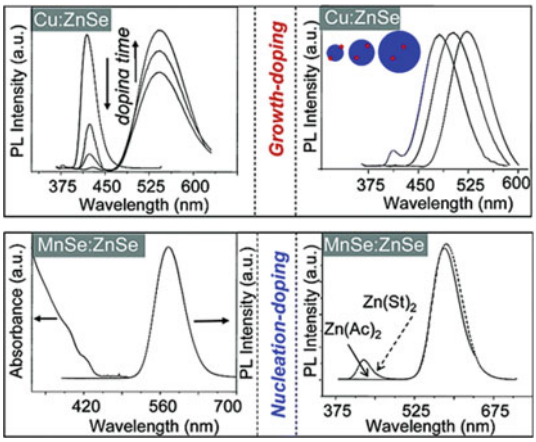

Fig. 2 Spectroscopic data for nucleation- and growth-doped QDs. Reproduced with permission from Ref. [26]

of MnSe: ZnSe nanocrystals. Zinc stearate necessitated a higher reaction temperature, leading in homogenous nucleation of ZnSe and a band gap PL. The main disadvantage of this approach is high-temperature annealing during shell formation, which causes dopants to escape and eventually leach out of the surface, leading to self-purification. This was specifically demonstrated in the instance of Cu-doped ZnSe QDs. The overcoating process carried out at a temperature of 210 °C effectively confined the dopants within the host lattice, thereby preventing any diffusion of dopants from the nucleus to the surface. Nevertheless, it was observed that upon annealing at 220 °C or above, the expulsion of dopants from the lattice occurred for all sizes. Furthermore, a notable reduction of the dopant photoluminescence was observed during annealing in the temperature range of 20–80 °C, as the overcoating temperature was raised. The process of overcoating ZnSe at elevated temperatures leads to a significant diffusion of Cu dopants toward the surface. Therefore, despite the prolonged endeavours of diverse groups on different systems to acquire internally doped QDs, the attainment of internally doped QDs has remained elusive.

The process of cation exchange reactions is extensively utilised in the doping of QDs, wherein the cation of the QDs is replaced with a dopant cation [28–30]. The extent of cation exchange can be tuned by varying the solution concentration of the incoming dopant cations and the type of ligands bound to them. These processes, however, are not trivial in semiconductor QDs, as evidenced by the large energies involved in doping. Furthermore, clustering or directional doping based on reactivity is inherent in this doping strategy. While the directed nature has been employed constructively to generate various hetero-structure topologies, clustering of dopant ions does not allow for homogenous impurity doping [31–33]. Another technique called diffusion doping, originally developed by Saha and colleagues, employs the self-purification mechanism instead of attempting to address it as an obstacle, a commonly perceived notion [34]. Using the SILAR (successive ionic layer adsorption and reaction) approach, a tiny magnetic core was over-coated with a thick shell

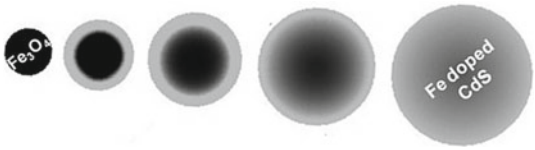

Fig. 3 Dopant diffusion into the CdS matrix to produce Fe-doped CdS QDs. Reproduced with permission from Ref. [34]

of the host semiconductor. High-temperature annealing of this core-shell arrangement causes the core to diffuse at the interface and into the semiconductor shell, eventually allowing it to diffuse out of the system. As illustrated in Fig. 3, the model system examined by Saha et al. comprised a Fe3O4 core overcoated with a CdS shell. The strained interface with a lattice mismatch of around 4% and high-temperature annealing causes core diffusion into the shell with a decrease in core size, eventually leading to uniformly doped QDs. Furthermore, the technique's generality and universality were shown for various dopant ions, such as M^{2+} (Fe^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+}). The efficiency of inside-out diffusion doping is determined by the core's capacity to enter the CdS lattice, followed by the metal ion's ability to diffuse within the lattice and the ease of cation exchange [35].

3 Factors Influencing Doping Efficiency

The distinctive electronic and optical characteristics of QDs (QDs) have generated considerable interest in contemporary times. Nanoscale semiconductor crystals exhibit significant potential across a diverse array of applications, encompassing electronics, optoelectronics, and biomedicine. The deliberate incorporation of impurities into QDs, commonly known as doping, is a pivotal technique in customising their characteristics and augmenting their efficacy. This discourse aims to investigate the variables that impact the efficacy of doping in QDs.

4 Role of Doping Precursor Concentration

The degree of dopant incorporation into the quantum dot lattice during synthesis is directly influenced by the concentration of the doping precursor. Elevated concentrations of doping precursors may augment the likelihood of the integration of dopant atoms into the quantum dot (QD) framework. On the other hand, reduced concentrations of precursors may yield a restricted quantity of dopants, thereby causing inadequate levels of doping. Attaining the targeted dopant concentration requires precise management of the precursor concentration throughout the synthesis procedure. The distribution and homogeneity of dopants within a quantum dot ensemble are influenced by the concentration of the doping precursor. An increased concentration of precursors frequently leads to a homogeneous dispersion of dopants within

the QDs. The homogeneity of the doped quantum dot (QD) population is a favourable attribute that facilitates the attainment of consistent and foreseeable characteristics. In contrast, reduced levels of precursor concentrations may give rise to dopants that are either spatially separated or clustered, thereby causing non-uniform doping and fluctuations in the performance of QDs. The control of size and composition in doped QDs can be influenced by the concentration of doping precursors. The growth kinetics and nucleation process of QDs in various synthesis methods, including colloidal synthesis, are influenced by the concentration of the precursor. Elevated concentrations of precursors typically facilitate accelerated rates of growth and increased sizes of QDs. Furthermore, it should be noted that the dopant material has the potential to impact the nucleation and growth patterns of the QDs. As a result, it becomes imperative to fine-tune the precursor concentration in order to attain the desired size and composition of the QDs. The optical and electronic characteristics of doped QDs are influenced by the concentration of the doping precursor. The concentration of dopant atoms within the QD lattice can exert an influence on optical properties, including emission wavelength and quantum yield. Elevated levels of precursor concentrations have the potential to augment the transfer of energy and the dynamics of charge carriers, ultimately resulting in alterations to the optical characteristics. The electronic characteristics of doped QDs (QDs), including energy levels and charge transport, are significantly influenced by the concentration of dopants, which ultimately affects the overall functionality of these QDs. The concentration of the doping precursor has an impact on the stability of QDs that have been doped, as well as their impurity effects. Elevated levels of dopant concentrations have the potential to introduce impurities and defects, thereby impacting the structural integrity and stability of QDs (QDs). Elevated precursor concentrations have the potential to cause non-uniform or inadequate doping, thereby leading to heightened impurity scattering or carrier recombination. These consequences can negatively impact the efficiency and stability of QDs.

Nair et al. have exhibited that the synthesis of metal- and nonmetal-codoped graphene QDs (GQDs) with improved optical properties can be achieved through the co-reaction of lignosulfonate (Fig. 4), which contains heteroatoms such as sulphur (S) and nitrogen (N) in its structure, and a metal precursor [36]. The GQDs derived from lignosulfonate synthesis exhibited notable blue fluorescence, displaying a substantial quantum yield of 23%. This phenomenon is ascribed to the presence of S and N doping, which was verified through the utilisation of X-ray photoelectron spectroscopy and Fourier transform infrared spectroscopy analyses. The lignosulfonate-derived GQDs were subjected to in situ doping to facilitate their modification. Additionally, a metal atom dopant was introduced to further engineer the GQDs, resulting in an improved quantum yield of 31%. This is noteworthy as it represents the highest quantum yield achieved for any GQDs derived from lignin.

A technique has been reported for the production of diluted magnetic semiconductor QDs (DMS-QDs) consisting of colloidal ZnO. This method involves the alkaline-activated hydrolysis and condensation of zinc acetate solutions in dimethyl sulfoxide (DMSO) [37]. The critical nuclei exhibit quantitative exclusion of dopants, while the nanocrystals' subsequent growth incorporates them in an almost isotropic

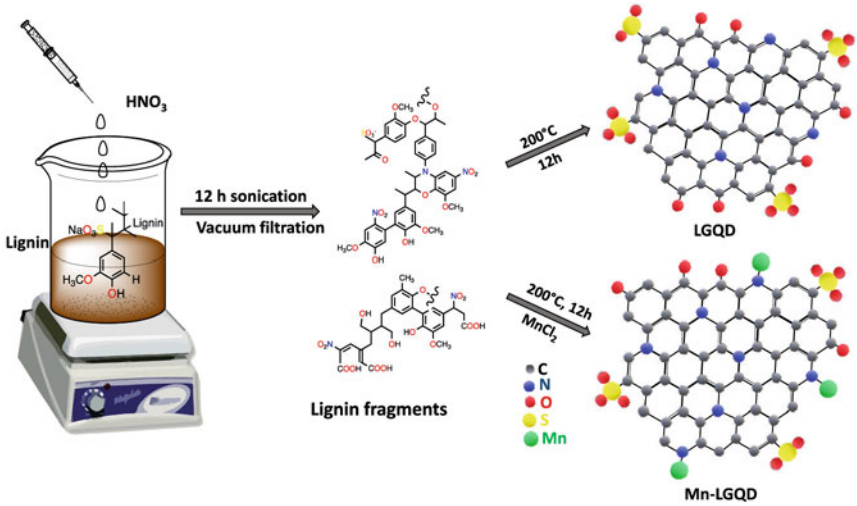

Fig. 4 A diagrammatic representation of the hydrothermal process for the synthesis of LGQDs and Mn-LGQDs. Reproduced with the permission from Ref. [36] © 2020 American chemical society

manner. The reduction in nanocrystal diameters that occurs as a result of doping can be elucidated by the Gibbs-Thompson relationship, which describes the correlation between lattice strain and crystal solubility. The process of synthesising graphene QDs (GQDs) involved the utilisation of femtosecond laser ablation in liquid, with laser induced graphene serving as the carbon source. The synthesis of Nitrogen-doped graphene QDs (N-GQDs) was achieved through the introduction of ammonia water into the graphene suspension [38]. The structure of GQDs/N-GQDs is composed of a graphitic core that exhibits oxygen and nitrogen functionalities, with a particle size that is smaller than 10 nm. This has been demonstrated through the use of various analytical techniques such as x-ray photoelectron spectroscopy, Fourier infrared spectrometer spectroscopy, and transmission electron microscopy. Lead halide perovskite QDs (PQDs) doped with transition metal cations are a promising group of materials for advanced photonic devices in the future. This is because of their fascinating properties induced by dopants and enhanced stabilities against photo-, moisture-, and thermal factors. The multifaceted nature of metal cation-doped PQDs in colloidal synthesis presents a challenging task when utilising conventional batch reactors. Bateni et al., employed a modular microfluidic platform that was equipped with a mobile spectral monitoring probe to investigate the kinetics and elucidate the chemical transformation mechanism of the rapid cation doping of cesium lead chloride QDs [39]. The authors additionally presented a design framework that enables accurate adjustment of the optical characteristics of QDs (QDs) through a post-synthetic doping mechanism in a flow-based system. The present study has developed a modular microfluidic platform that, in conjunction with facile doping chemistry, can facilitate the uninterrupted production of metal cation-doped PQDs possessing optoelectronic properties tailored to specific applications.

4.1 Role of Reaction Temperature and Time

The production of doped QDs (QDs) necessitates a meticulous equilibrium of multiple parameters to attain the intended characteristics. The reaction temperature and reaction time are significant parameters that impact the properties and efficacy of doped QDs. The present discourse aims to examine the importance of reaction temperature and duration and their influence on the production of doped QDs.

The incorporation of dopants into the quantum dot lattice during synthesis is directly influenced by the reaction temperature and time. Elevated temperatures typically facilitate the diffusion of dopant precursors into the quantum dot (QD) architecture, resulting in heightened levels of doping. Excessive temperatures can lead to agglomeration or thermal decomposition, which can have adverse effects on the quality and consistency of the doped QDs. Similarly, the duration of the reaction permits ample time for the inclusion of dopant atoms, and extended reaction times can result in increased levels of doping given suitable temperature conditions. The manipulation of reaction temperature and duration exerts a notable impact on the regulation of size and composition of doped QDs. Elevated temperatures frequently facilitate accelerated nucleation and growth kinetics, leading to the formation of comparatively larger QDs. In contrast, reduced temperatures could hinder the developmental mechanism and result in the formation of diminutive QDs. The overall size distribution and size homogeneity of the doped QDs are influenced by the reaction time in a similar manner. The manipulation of temperature and reaction time allows for precise regulation of the dimensions and constitution of QDs, which is imperative in customising their optical and electronic characteristics. The crystal structure and stability of doped QDs are also influenced by the reaction temperature and duration. The kinetics of nucleation and growth processes, as well as the crystal structure of QDs, is impacted by temperature. Elevated temperatures have the potential to facilitate the process of crystal growth and enhance the degree of crystallinity, thereby resulting in heightened stability. Nevertheless, elevated temperatures have the potential to cause imperfections or separation of phases. The growth dynamics are influenced by the reaction time, which provides adequate time for the development of a clearly defined crystal structure. Achieving stable and structurally well-defined doped QDs (QDs) requires the optimisation of temperature and reaction time.

The manipulation of reaction temperature and time is a crucial aspect in customising the optical and electronic characteristics of doped QDs. The manipulation of reaction conditions enables regulation of the quantum yield, energy levels, and emission wavelength of the QDs. Elevated temperatures have the potential to facilitate energy transfer mechanisms and the dynamics of charge carriers, leading to alterations in optical characteristics. Furthermore, the duration of the reaction time has an impact on the creation of imperfections and obstructions within the QDs, potentially altering their electronic characteristics, including the movement of charge carriers and the rate of recombination.

The kinetics of the synthesis process and the controllability of doped QDs are influenced by the reaction temperature and time. Incomplete doping or undesired

side reactions may occur due to rapid temperature changes or shorter reaction times. Conversely, extended reaction times or reduced temperatures may facilitate the occurrence of secondary reactions or undesired contaminants. Attaining the ideal reaction parameters in terms of temperature and duration facilitates superior regulation of the synthesis procedure, culminating in the production of doped QDs of exceptional quality.

4.2 Role of QD Size and Shape

The optical, electronic, and chemical properties of QDs (QDs) are significantly influenced by their size and shape. The size and shape parameters are critical factors in determining the doping efficiency and resulting characteristics of synthesised doped QDs. The present discourse aims to investigate the significance of quantum dot dimensions and configurations in the production of doped QDs.

The doping efficiency during synthesis is influenced by the size of QDs. In general, QDs that is smaller in size exhibit a greater surface-to-volume ratio, thereby augmenting the interaction between the dopant precursor and the surface of the quantum dot. The enhanced surface interaction promotes the integration of dopant atoms into the lattice of the quantum dot, leading to an enhancement in the efficiency of doping. In contrast, QDs of greater size exhibit a diminished surface-to-volume ratio, thereby limiting the surface area accessible for dopant integration and potentially resulting in decreased doping efficacy.

The doping process is also influenced by the shape anisotropy of QDs, which may take on various forms such as spherical, rod-shaped, or plate-like. The surface energies and reactivity of crystallographic facets on various quantum dot shapes can differ. The surface characteristics have the potential to impact the adsorption and diffusion of dopant precursors in the course of synthesis. The phenomenon of preferential doping on specific facets due to shape anisotropy can result in a non-uniform distribution of doping within the ensemble of QDs. Comprehending the doping behaviour that is contingent on the shape is of utmost importance in regulating the characteristics of doped QDs.

The electronic and optical properties of QDs are directly impacted by their size, thereby exerting an influence on the doping behaviour. As the size of the quantum dot diminishes, the quantum confinement effect becomes increasingly conspicuous, resulting in distinct energy levels and adjustable optical characteristics. The size-dependency of QDs significantly impacts their energy levels and bandgap, thereby influencing the arrangement of dopant energy levels in the QD band structure. The properties of QDs that are dependent on their size are of utmost importance in the optimisation of doping techniques and the attainment of desired functionalities. The carrier dynamics, which encompass charge carrier generation, recombination, and transport, are notably impacted by the dimensions of QDs. The discrete energy levels and reduced non-radiative recombination pathways of smaller QDs typically result in faster carrier relaxation and radiative recombination rates. The utilisation of doping

has the potential to modify the dynamics of carriers through the alteration of charge transfer processes and the modification of energy states. Comprehending the carrier dynamics that are dependent on the size is of utmost importance in order to enhance doping techniques and achieve the highest possible efficiency of doped QDs. The stability and surface passivation requirements of QDs are also influenced by their size. QDs with larger dimensions exhibit a greater density of surface defects and trap states, owing to their increased surface area (Table 1). The introduction of impurities or dopant-induced defects due to doping can have an impact on the surface passivation requirements. QDs exhibit a greater surface-to-volume ratio, which necessitates the implementation of more efficient surface passivation techniques to counteract surface-related recombination and improve their stability. The management of size-dependent stability and surface passivation is a crucial factor in attaining sustained efficacy and robustness of doped QDs.

5 Strategies to Optimize Doping Efficiency

Various approaches can be utilised to enhance doping efficacy in QDs (QDs) for optimal performance. The present strategies are centred on augmenting the integration of dopants into the quantum dot lattice and attaining the targeted levels of doping. The following are several fundamental methods for enhancing doping efficiency:

- The selection of a suitable dopant material is a critical factor in achieving optimal doping efficiency. Efficient energy level alignment and charge transfer can be achieved by selecting a dopant material that possesses electronic properties that are compatible with those of the QD matrix. Furthermore, it is imperative that the dopant material demonstrates favourable solubility within the quantum dot (QD) matrix and maintains stability throughout the doping procedure. Thorough evaluation of the dopant material characteristics is crucial for the effective integration of said material into the quantum dot lattice.
- In the field of engineering, it is imperative to optimise the doping precursor in order to enhance the efficiency of doping. The process entails the careful selection of dopant precursors with high reactivity and favourable thermodynamics for the doping reaction. Advanced engineering methodologies, such as the manipulation or creation of ligands, can be utilised to amplify the responsiveness and durability of the precursor, thereby facilitating its interaction with the quantum dot surface and promoting effective doping.
- The optimisation of different process parameters can enhance the efficiency of doping. The aforementioned parameters encompass temperature, duration of reaction, concentration of precursor, and atmosphere of reaction. Optimising these parameters according to the particular doping scheme and target doping concentrations can improve the integration of dopants into the quantum dot crystal structure. Modulating the temperature and reaction duration can exert an impact on the

Table 1 Sum up the major strategies and their examples

Strategies	Description	Examples/considerations	References
Selection of suitable dopant material	Choose dopant materials with compatible electronic properties to achieve desired energy level alignment and charge transfer	ZnSe QDs doped with Mn^{2+}, CdSe QDs doped with Cu^{+} ions	[40]
Precursor engineering	Modify dopant precursors to enhance reactivity, stability, and solubility in the QD matrix, improving dopant incorporation efficiency	Ligand design, precursor synthesis optimization, chelating agents for stabilization	[41, 42]
Control over doping process parameters	Optimize temperature, reaction time, precursor concentration, and atmosphere to enhance diffusion kinetics and achieve desired doping levels	Higher temperatures for increased diffusion, longer reaction times for improved incorporation	[1, 43, 44]
Surface engineering and passivation	Employ surface ligands, shell layers, or surface treatments to passivate QD surface, reducing surface defects and improving doping efficiency	Ligand exchange, shell growth, surface passivation techniques	[45, 46]
Core-shell doping	Utilize core-shell QDs to selectively dope the core or shell regions, enabling precise control over doping levels and charge transfer processes	CdSe/CdS core-shell QDs doped with transition metal ions	[47]
Hybrid doping approaches	Combine multiple doping techniques, such as ion implantation, chemical doping, or cation exchange, to enhance doping efficiency synergistically	Sequential doping processes, simultaneous doping with different dopants	[48]
Characterization and feedback	Employ various characterization techniques (e.g., spectroscopy, microscopy, electrical measurements) to assess doping efficiency and optimize	Photoluminescence spectroscopy, TEM/SEM imaging, current-voltage characteristics	[49, 50]

diffusion and kinetics of dopant incorporation, whereas fine-tuning the precursor concentration guarantees the attainment of the intended doping levels.

- The efficiency of doping can be hindered by non-radiative recombination or impurity scattering caused by surface defects and trap states. Therefore, surface engineering and passivation are crucial in addressing this issue. The implementation of proficient surface engineering and passivation methodologies can alleviate these concerns and amplify the efficacy of doping. The implementation of surface ligands, shell layers, or surface treatments can serve as a means of passivating the surface of QDs (QDs), thereby reducing surface defects and enhancing carrier

confinement. The implementation of surface engineering techniques enhances the general stability, optical characteristics, and doping efficacy of QDs.

- Core-shell doping is a technique that can be utilised to improve doping efficiency in QDs (QDs). Specifically, the efficiency can be enhanced by selectively doping either the core or shell regions of the QDs. The utilisation of a core-shell structure enables a regulated milieu for the inclusion of dopants, thereby facilitating meticulous modulation of the doping levels. Through the strategic introduction of dopants into either the core or shell, it is feasible to enhance charge transfer mechanisms, mitigate the emergence of dopant-induced flaws, and enhance the efficacy of doping.
- Hybrid doping approaches refer to the combination of various doping techniques with the aim of optimising their effectiveness. Multiple dopants can be introduced or the doping process can be enhanced through the use of sequential or simultaneous doping techniques. The simultaneous utilisation of ion implantation, chemical doping, or cation exchange methods can result in a synergistic enhancement of doping efficiency, as each technique can leverage its unique advantages.
- The utilisation of characterization techniques, such as spectroscopy, microscopy, and electrical measurements, can offer significant insights into the effectiveness of doping. The assessment of various doping strategies and optimisation of the doping process can be achieved by monitoring the optical and electronic properties of doped QDs (QDs). The iterative enhancement of doping efficiency is facilitated by the approach that is driven by feedback.

6 Applications of Doped QDs

6.1 Bioimaging

QDs that have been doped have been utilised in diverse areas such as bioimaging and biosensing. The following are several significant implementations:

The utilisation of doped QDs as imaging agents in biological systems is highly advantageous due to their distinctive optical properties in the field of bioimaging. Due to their diminutive size, exceptional photostability, and customizable emission spectra, they are well-suited for a diverse array of imaging methodologies, including fluorescence microscopy and in vivo imaging. Through the conjugation of doped QDs with targeting molecules, scientists are able to selectively label and monitor different cellular components, biomolecules, or particular disease markers within living organisms. The utilisation of doped QDs in biosensing applications based on Fluorescence Resonance Energy Transfer (FRET) is feasible. Förster resonance energy transfer (FRET) is a phenomenon of energy transfer that transpires non-radiatively between a donor fluorophore and an acceptor fluorophore when they are in close proximity. Through the utilisation of Förster resonance energy transfer (FRET), scientists can effectively track and analyse targeted biochemical reactions or interactions by combining a doped quantum dot with an acceptor fluorophore.

This technique allows for the detection of biomolecular events with exceptional sensitivity and spatial resolution. Proximity-based assays employ doped QDs to measure the spatial proximity of target molecules. The detection and quantification of target molecules can be achieved by conjugating target-specific molecules, such as antibodies or aptamers, to doped QDs. This approach relies on the proximity-induced changes in the optical properties of the QDs. This approach is useful for various applications, including protein-protein interaction studies and the detection of biomarkers in clinical samples. The application of doped QDs in single-molecule tracking provides exceptional photostability, facilitating the extended and precise visualisation of singular molecules. This property is particularly useful in single-molecule tracking experiments, where the movement and behavior of individual biomolecules (such as proteins or nucleic acids) can be monitored in real-time. Through the utilisation of doped QDs, scientists can acquire significant knowledge regarding the dynamics, interactions, and cellular processes of these molecules. The integration of doped QDs with other imaging modalities, such as magnetic resonance imaging (MRI) or computed tomography (CT), can result in the development of multimodal imaging agents. These hybrid agents can provide complementary information and enable enhanced imaging capabilities, such as combining the high-resolution imaging of doped QDs with the anatomical information from MRI or CT, leading to improved diagnosis and monitoring of diseases. In general, doped QDs have demonstrated their versatility and significance in the fields of bioimaging and biosensing, providing improved sensitivity, photostability, and multiplexing abilities for various biological investigations. A facile hydrothermal technique was employed to synthesise nitrogen-sulfur doped graphene QDs (NS-GQDs). The stability of fluorescence signals exhibited by NS-GQDs remains high even in the presence of diverse metal ions [51]. Aspartic acid and cysteine were employed as carbon precursors and heteroatomic sources of nitrogen and sulphur, respectively. The NS-GQDs that were generated exhibited a quantum yield of up to $19.3 \pm 1.7\%$ and a peak emission of 480 nm when excited by 400 nm. The results depicted in Fig. 5a demonstrate a noteworthy decrease in the fluorescence of Pdots within cells upon the introduction of 200 μM Cu^{2+}. This finding suggests that Cu^{2+} has a quenching effect on Pdots in the context of bioimaging. On the other hand, it was observed that the fluorescence imaging ability of cells stained with NS-GQDs remained unaffected upon treatment with 200 μM Cu^{2+}, as depicted in Fig. 5b. The findings indicate that the NS-GQDs that were created exhibit exceptional resistance to metal ions in the context of bioimaging.

6.2 Optoelectronics

The unique properties and potential applications of doped QDs (QDs) have garnered a lot of attention in optoelectronics. The wide applicability of QDs and their ongoing works have been tabulated in Table 2. Researchers have found that by doping or

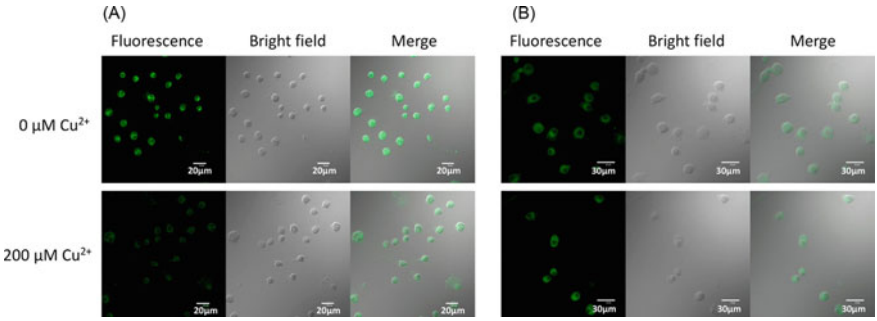

Fig. 5 LSCM images of RAW 264.7 cells incubated with **a** 10.0 μg/mL Pdots, **b** 1.0 mg/mL NS-GQDs with 200 μM Cu^{2+}. Reproduced with the permission from Ref. [51] © 2019 American chemical society

introducing impurities into the QDs, they can alter their electronic and optical properties, expanding their potential for use in optoelectronic devices. Some examples of where doped QDs have found use in optoelectronics are as follows:

Doped QDs can function as emitters in light-emitting diodes (LEDs). Researchers have found that they can tune the QDs' emission wavelength across the visible and near-infrared spectrum by adjusting their size and composition. QDs are promising candidates for next-generation display technologies because their efficiency, stability, and colour purity can be further improved by doping them with specific impurities.

Doped QDs may improve the efficiency of solar cells, which is important in the field of photovoltaics. It is possible to increase the solar cell's absorption range by incorporating doped QDs into the active layer. Dopants can also help with charge separation and transport, which boosts the solar cell's overall efficiency.

Doped QDs are an active material that can be used in photodetectors. Scientists can design QDs to be exceptionally sensitive to certain colours of light by adjusting the bandgap and the amount of doping. This paves the way for the creation of photodetectors with enhanced responsivity, rapid response, and minimal noise.

When it comes to lasers, doped QDs can be used as gain media. In order to achieve population inversion and stimulated emission, the dopants introduce energy levels within the QD bandgap. Doped QD lasers are useful for telecommunications, spectroscopy, and medical diagnostics because they can operate at a wide range of wavelengths, from the near-infrared to the visible and even the mid-infrared.

When it comes to quantum computing and information processing, doped QDs can serve as qubits. Implementing quantum logic operations requires manipulating electron spins and charge states in the QDs, which is made possible through the controlled introduction of dopants. Doped QDs have the potential to contribute to the growth of quantum technologies by enabling scalable and coherent qubit systems.

Table 2 Various applications of QDs device making and fabrications

Application	Description	Benefits	References
Light-emitting diodes	Doped QDs serve as emitters in LEDs, enabling tunable and efficient displays with enhanced color purity	Wide color gamut, high efficiency, long operational lifetime, solution processability	[52]
Photovoltaics	Doped QDs enhance the absorption range and charge transport in solar cells, improving overall efficiency	Broadband light absorption, enhanced charge collection, compatibility with flexible substrates	[53–55]
Photodetectors	Doped QDs enable the development of highly sensitive and fast photodetectors with low noise characteristics	High responsivity, wide spectral range, low dark current, ultrafast response time	[56]
Lasers	Doped QDs act as gain media, facilitating population inversion and stimulated emission for lasers in various wavelengths	Wavelength tunability, low threshold current, high quantum efficiency, compact size	[57]
Quantum computing	Doped QDs can be used as qubits for quantum computing and information processing, leveraging controlled electron spins and charge states	Scalability, long coherence times, high-fidelity operations, interfacing with solid-state platforms	[58]
Biological imaging	Doped QDs offer excellent optical properties for bioimaging, including high brightness, photostability, and tunable emission wavelengths	Bright and stable fluorescence, multiplexed imaging capabilities, targeted molecular imaging, high signal-to-noise ratio	[13]
Sensing applications	Doped QDs are utilized for sensing applications, such as chemical and biological sensing, due to their high sensitivity and selectivity	High sensitivity and selectivity, label-free detection, real-time monitoring, miniaturization potential	[59]
Display technologies	Doped QDs enable the development of vibrant and energy-efficient displays with wide color gamut and high contrast ratios	Vivid and accurate colors, energy efficiency, high contrast ratio, flexible and transparent displays	[60, 61]

(continued)

Table 2 (continued)

Application	Description	Benefits	References
Optical data storage	Doped QDs have potential applications in optical data storage due to their stable and controllable optical properties	High storage density, long-term data retention, fast data writing and reading, compatibility with existing technology	[62]
Energy-efficient lighting	Doped QDs can be used in solid-state lighting to achieve energy efficiency and long lifetimes, such as in quantum dot-based down-converters for LEDs	High color quality, energy efficiency, long operational lifetime, warm white lighting	[63, 64]

6.3 Photovoltaics

The utilisation of doped QDs is currently being extensively investigated for their potential application in photovoltaics [65–68], with a particular focus on enhancing light absorption and overall conversion efficiency. The bandgap of QDs can be precisely adjusted to enhance the absorption of a wider range of wavelengths in the solar spectrum by introducing various materials or impurities. Furthermore, it has been observed that QDs that have been doped possess the capability to produce numerous excitons from a solitary high-energy photon. This occurrence is commonly referred to as multiple exciton generation (MEG) and has the potential to augment the effectiveness of solar cells. Moreover, the introduction of precise impurities can result in the formation of intermediate bands within the bandgap of QDs, which facilitates the absorption of photons possessing energies lower than the material's bandgap. The utilisation of intermediate band solar cells presents a potentially auspicious pathway towards achieving enhanced efficiency in the conversion of solar energy. In addition, doped QDs have the potential to serve as sensitizers in the context of QDs sensitised solar cells [69–71], wherein they are capable of absorbing photons and subsequently transferring the resulting electrons to a photoactive material. This methodology enables the utilisation of economical and solution-deposited substances while upholding elevated conversion efficacy. Ongoing investigation and innovation in the application of doped QDs exhibit significant promise in propelling the domain of photovoltaics forward.

The synthesis of N, S-doped carbon QDs (N, S-CQDs) was reported through a straightforward hydrothermal treatment approach utilising ascorbic acid and ammonium persulfate as reagents [72]. The enhancements in performance and the removal of the light-soaking phenomenon in ZnO:N, S-CQDs cells are ascribed to the passivation of surface defects in ZnO by N, S-CQDs (Fig. 6). This conclusion is supported by fluorescence spectroscopy and scanning Kelvin probe microscopy. The power conversion efficiency of cells with N, S-CQDs-modified ZnO ETL was observed to be 9.31%, indicating a significant improvement compared to the reference ZnO cells.

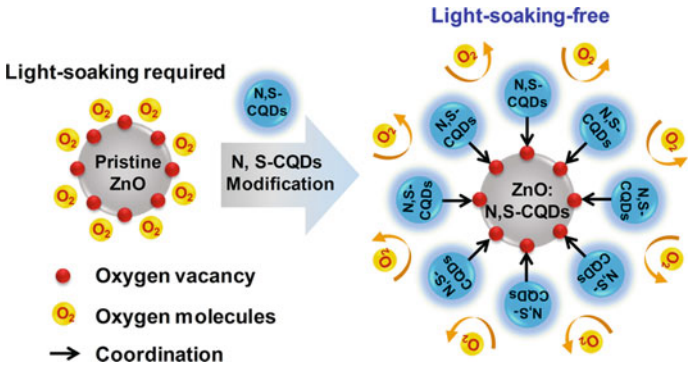

Fig. 6 The mechanism for the passivation of surface defects on ZnO nanoparticles through the utilisation of N, S-functionalized carbon QDs (CQDs). Reproduced with the permission from Ref. [72] © 2019 American chemical society

Si QDs (QDs) were synthesised in SiO2 using a co-sputtering technique, resulting in multiple layers with a narrow size distribution. The present study investigates the structural, electrical, and optical characteristics of multilayer films composed of Si QDs (QDs) and SiO2. The films were prepared by introducing varying concentrations of boron (B) during the sputtering process [73]. It was observed that the intensity of photoluminescence (PL) decreased as the concentration of boron increased. Yang et al. conducted a study in which they produced a range of nitrogen-doped carbon QDs (N-CQDs) through hydrothermal synthesis at varying temperatures, utilising Eichhornia crassipes (ECs) as the source material [74]. The researchers conducted an experimental fabrication of N-CQDs sensitised solar cells, resulting in lower power conversion efficiency when compared to state-of-the-art devices that are based on silicon, polymer, dye, CdS, CdSe, or perovskite.

6.4 Catalysis

Doped QDs have exhibited considerable potential in the domain of catalysis, wherein they can augment the efficacy and specificity of diverse chemical reactions. Several potential uses of doped QDs in catalysis have been identified. The utilisation of doped QDs as photocatalysts is a promising approach for facilitating chemical reactions through the conversion of light energy. The process of doping allows for accurate manipulation of the electronic configuration, thereby facilitating the production and transmission of charge carriers. The aforementioned ability amplifies the effectiveness of photocatalytic processes, such as the division of water for the purpose of producing hydrogen or the breakdown of pollutants. QDs, upon doping with particular atoms or functional groups, exhibit remarkable catalytic activity and selectivity for diverse reactions in the realm of heterogeneous catalysis. The process of doping

induces modifications in the surface characteristics and electronic configuration, thereby facilitating improved adsorption, activation, and reaction kinetics. The utilisation of doped QDs has been investigated for catalysing various reactions, including carbon dioxide reduction, nitrogen fixation, and organic transformations. The utilisation of doped QDs as electrocatalysts has been observed in facilitating electrochemical reactions, specifically oxygen reduction and hydrogen evolution [75–77]. This process is known as electrocatalysis. The process of doping has the potential to enhance the electronic structure, thereby facilitating charge transfer kinetics and augmenting catalytic performance. QDs that have been doped exhibit enhanced electrocatalytic performance in comparison to conventional catalysts, thereby presenting promising prospects for utilisation in fuel cells, batteries, and electrolyzers. Doped QDs have the ability to imitate the catalytic activity of enzymes, which is commonly referred to as artificial enzyme mimetics or nanozymes. The catalytic properties of enzymes, such as oxidase, peroxidase, or catalase-like activities, can be emulated by doping QDs with particular ions or functional groups [78, 79]. Nanozymes present several benefits, including enhanced stability, customizable properties, and economical feasibility in contrast to their natural enzyme counterparts. These attributes create opportunities for their implementation in various domains, such as biosensing, environmental restoration, and medical research.

Prekodravac et al. presented a rapid and efficient method for synthesising nitrogen-doped carbon QDs in an aqueous medium without the use of metal catalysts. This was achieved through the utilisation of a microwave reactor [80]. A synthesis was conducted on a glucose water solution utilising a microwave reactor under low temperature and applied microwave power for a duration of only 1 min. The N-doped carbon QDs that were synthesised exhibit noteworthy photocatalytic efficacy in eliminating hazardous organic dye (Rose Bengal) when subjected to visible light radiation. A significant degradation of approximately 93% of the dye is attained within a brief period of 30 min of radiation. The synthesis of fluorescent nitrogen doped carbon dots (N-CDs) was discussed in a report, using a straightforward hydrothermal method [81]. The N-CDs exhibit remarkable catalytic efficacy in the sodium borohydride-mediated reduction of methylene blue. Elsewhere, a report was made on the green synthesis of fluorescent nitrogen-doped carbon dots (N-CDs) utilising *Actinidia deliciosa* (A. deliciosa) fruit extract as a carbon precursor and aqueous ammonia as a nitrogen dopant [82]. Chandrasekaran et al. have documented a straightforward and environmentally-friendly hydrothermal method for synthesising nitrogen-doped carbon dots (N-CDs) utilising *C.grandis* and aq.NH3 as carbon and nitrogen sources, respectively [83]. The catalytic activity of NaBH4 in the reduction of methyl orange was affected by the synthesised N-CDs. The rate constant of the reduction process of organic dye, specifically methyl orange, by NaBH4 in the presence of the synthesised green catalyst was also ascertained.

7 Prospects for Future Developments and Applications

The potential for advancements and utilisation of doped QDs (QDs) in the future is exceedingly auspicious. The following are prospective domains of progress.

Doped QDs (QDs) can be customised to demonstrate distinct optical and electronic characteristics, rendering them highly suitable for advanced optoelectronic devices. Enhancements in synthesis methodologies have the potential to facilitate the creation of lasers, LEDs, and photodetectors based on QDs, which exhibit superior efficiency, colour purity, and stability.

Doped QDs (QDs) exhibit significant promise in sensing applications owing to their exceptional sensitivity and selectivity. The optical properties of materials can be adjusted to enable the detection of particular molecules or ions by introducing precise dopants. Prospective advancements could result in the production of exceedingly responsive and easily transportable sensors based on QDs (QDs) for the purposes of monitoring the environment, diagnosing medical conditions, and ensuring food safety.

Doped QDs (QDs) have the potential to significantly contribute to the advancement of renewable energy technologies through energy harvesting and storage. By enhancing their electronic characteristics, they can be employed in high-performance photovoltaic cells for the conversion of solar energy into electrical energy. Moreover, the utilisation of doped QDs (QDs) can be investigated for the purpose of energy storage in various applications, including supercapacitors and electrochemical reactions as catalysts.

Doped QDs (QDs) have garnered significant interest as contrast agents in bioimaging and as targeted drug delivery systems for biomedical applications. The distinctive optical characteristics of nanoparticles, in conjunction with surface alterations, facilitate the accurate visualisation of biological structures and the regulated dispensation of therapeutic agents. Subsequent progressions may potentially result in the creation of diagnostic and therapeutic instruments that are safer and more effective in the field of medicine.

Doped QDs (QDs) exhibit potential for utilisation in quantum computing and quantum information processing, as they possess the capability to confine and manipulate individual charges and spins. Advancements in dopant engineering and control have the potential to facilitate the achievement of quantum computing architectures that are both scalable and resilient.

Doped QDs (QDs) have potential for utilisation in environmental applications, specifically in photocatalysis for the purpose of water purification or pollutant degradation. Moreover, these can be incorporated into lighting systems that are designed to be energy-efficient, thereby mitigating energy usage and environmental repercussions. Prospective progressions may facilitate the creation of more environmentally friendly technologies that entail diminished resource consumption and enhanced efficacy.

In general, the potential applications and advancements of doped QDs are extensive and offer significant promise in diverse scientific and technological domains.

Further investigation, refinement of manufacturing techniques, and examination of innovative additives and structures will facilitate the realisation of their complete potential and broaden their pragmatic implementations.

8 Present Market of Doped QDs

The market for doped QDs (QDs) is still in its early stages but is projected to experience substantial growth in the coming years. The unique characteristics and potential applications of doped QDs (QDs) are the primary drivers behind the increasing demand for them across diverse industrial sectors. The market for QDs (Fig. 7), including doped QDs, is expected to grow significantly in the coming years. Several market research reports indicate that the QDs market is anticipated to attain a significant valuation in the billions by the year 2026, exhibiting a compound annual growth rate (CAGR) exceeding 20%. The growth in question is being propelled by several factors, including but not limited to the escalating demand for displays that are energy-efficient, the progress made in healthcare imaging technologies, and the increasing acceptance of sensors that are based on QDs. While it's challenging to provide specific market figures, here are some key insights into the current market of doped QDs:

Doped QDs (QDs) are increasingly being utilised in the optoelectronics sector, specifically in display technologies like quantum dot LED (QLED) displays. QLED displays provide several benefits, including superior colour precision, a broad range of colours, and low power consumption. Doped QDs are progressively gaining popularity in the realm of consumer electronics, encompassing devices such as televisions, smartphones, and monitors.

Doped QDs (QDs) are employed as fluorescent markers in biomedical imaging owing to their exceptional optical characteristics, including high luminosity and

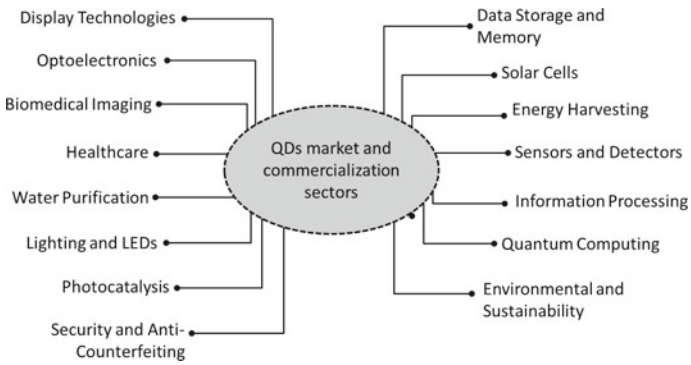

Fig. 7 Different types of application areas for QDs based products

resistance to photobleaching. They offer enhanced imaging capabilities for applications in biological research, diagnostics, and drug delivery. Ongoing research and commercialization efforts have identified the biomedical imaging sector as a significant market for doped QDs.

Energy and Solar Cells: Doped QDs hold promise in the field of energy conversion and storage. The tunable bandgap and high absorption efficiency of these materials have rendered them a subject of exploration for their potential application as constituents of next-generation solar cells. Doped QDs offer the potential for increased solar cell efficiency and reduced production costs, driving their adoption in the renewable energy sector.

Sensors and Detectors: Doped QDs are being developed for various sensing applications. They exhibit high sensitivity, selectivity, and stability, making them suitable for environmental monitoring, biosensing, and chemical detection. Doped QD-based sensors and detectors have the potential to provide rapid, accurate, and cost-effective solutions for diverse industries.

Research and Development: The market for doped QDs is also supported by ongoing research and development activities. Researchers are exploring new doping strategies, optimizing synthesis techniques, and investigating novel applications. This continuous innovation fuels the growth of the doped QD market and opens up possibilities for emerging applications.

It's important to note that the market for doped QDs is dynamic and evolving, with ongoing technological advancements and increasing commercialization efforts. The integration of doped QDs into various industrial applications is anticipated to result in an increase in market size and revenue.

9 Conclusion

To summarise, the synthesis strategies utilised for doped QDs comprise a variety of methods with the objective of integrating dopants into the QD lattice to attain specific electronic and optical characteristics. The aforementioned tactics encompass codoping, post-synthetic doping, ion exchange, alloying, and core-shell architectures. The process of codoping entails the concurrent introduction of multiple dopants during the synthesis of QDs, thereby enabling accurate regulation of both dopant concentration and combination. The process of post-synthetic doping pertains to the introduction of dopants onto pre-synthesized QDs through surface modification or ligand exchange procedures. The process of ion exchange entails the substitution of the initial cations or anions present in the quantum dot lattice with dopant ions. The process of alloying involves the integration of distinct semiconductor materials, along with the inclusion of dopants during the alloying procedure. The utilisation of core-shell structures allows for the confinement of doped QDs within a safeguarding shell, thereby facilitating the regulation of dopant diffusion and associated characteristics.

The implementation of synthesis strategies provides a malleable and adaptable approach to customise the characteristics of doped QDs to meet particular utilisation

requirements. The optimisation of doped QDs for various applications such as optoelectronics, photovoltaics, sensing, and bioimaging can be achieved by manipulating dopant concentration, distribution, and combination, as per the research findings. In summary, the implementation of synthesis strategies involving doped QDs has resulted in notable progressions within the realm of nanotechnology and nanomaterials. The deliberate incorporation of impurities into QDs has presented novel opportunities for the manipulation of their optical and electronic characteristics, resulting in improved functionalities and innovative applications. Further investigation in this domain exhibits potential for enhancing these amalgamation approaches, broadening the scope of dopants and substrates, and unleashing the complete potential of doped QDs in various domains.

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Quantum Dots Based Nanocomposites

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Abstract This chapter explores the topic of quantum dots-based nanocomposites, providing a synopsis of their fabrication, characterisation, and varied practical applications. The unique qualities of quantum dots as building blocks are discussed, along with their relevance in fields such as photovoltaics, optoelectronics, biomedicine, and environmental monitoring. This chapter focuses on the cutting-edge techniques used to create them and highlights the importance of adapting their size, shape, and composition to a variety of uses. Characterization methods are presented in detail for understanding charge transfer, energy transfer, and interfacial interactions in nanocomposites. Examples of their use in solar cells, LEDs, targeted medication delivery, bio imaging, and environmental clean-up demonstrate their potential for far-reaching influence. Future directions for realising the full potential of quantum dots-based nanocomposites in advancing nanotechnology towards a sustainable and

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efficient future are also discussed in this chapter, along with obstacles relating to safety, scalability, and technological hurdles.

Keywords Quantum dots · Bio imaging · Synthesis · Electrochemical · Characterization

1 Introduction

Quantum dots refer to semiconductor particles at the nanoscale level that exhibit distinct characteristics as a result of confinement effects in the quantum realm [1]. The optical behaviour of quantum dots is known to be dependent on their size, which is considered to be a remarkable property. The confinement of electrons and holes within the dot gives rise to the "quantum size effect" phenomenon [2]. The energy levels of charge carriers become quantized and discrete as the quantum dot size decreases. As a result, the bandgap, which governs the amount of energy needed for an electron to move from the valence band to the conduction band, exhibits an upward trend as the size of the dot decreases [3]. The tunability of the band gap in quantum dots results in a distinctive characteristic, namely the capacity to produce light emissions of varying hues contingent upon their dimensions. Through the manipulation of synthesis parameters, researchers possess the ability to precisely engineer quantum dots, resulting in the emission of light spanning the visible spectrum, as well as the ultraviolet and infrared regions [4–6]. The high photoluminescence quantum yield is a notable characteristic of quantum dots. Quantum dots exhibit high efficiency in emitting light when stimulated by photons, rendering them a desirable choice for utilisation in optoelectronic devices like light-emitting diodes (LEDs) and displays [7]. The narrow spectral linewidths exhibited by these entities render them appropriate for deployment in biological imaging and sensing applications, wherein the attainment of accurate and luminous fluorescence signals is of paramount importance [8]. Quantum dots exhibit remarkable photostability, indicating their ability to retain their optical characteristics for extended durations without notable deterioration. The maintenance of stability is of paramount significance in imaging and sensing applications, given the indispensable requirement of precise and uniform measurements [9].

The noteworthy characteristics of quantum dots include their electrical properties. Quantum dots are characterised by discrete energy levels owing to their diminutive size, which is commonly known as "Coulomb blockade" [10]. The aforementioned characteristic of the property facilitates the manipulation of individual electrons or holes confined within the dot, thereby rendering them suitable for utilisation in quantum computing and single-electron transistors [11]. Moreover, quantum dots manifest distinct electronic interactions commonly referred to as "quantum confinement effects". The aforementioned impacts lead to alterations in the electronic band configuration and the emergence of distinct energy states. Quantum dots' electronic characteristics can be manipulated through impurity doping, which can introduce supplementary energy levels and alter their conductivity [12]. The properties of

quantum dots are also influenced by their size and shape [13]. The bandgap and consequent emission colour of a quantum dot are determined by its size. In addition, quantum dots exhibit diverse morphologies, including but not limited to spherical, rod-shaped, and tetrapodal, which can significantly impact their electronic and optical properties. The diverse range of shapes exhibited by quantum dots enables the precise modulation of their characteristics, thereby facilitating the development of tailored materials utilising quantum dots for targeted functionalities [14].

Quantum dots possess distinctive characteristics and adjustability, providing numerous benefits and serving a wide range of purposes in diverse domains. Nanoparticles composed of semiconducting materials have surfaced as a groundbreaking technology, offering a multitude of advantages spanning from cutting-edge imaging and sensing capabilities to energy acquisition and quantum computation [15]. Quantum dots possess a significant advantage in terms of their optical properties, which can be adjusted based on their size [16]. Through the manipulation of quantum dot dimensions and constituents, researchers are able to meticulously design the emission wavelength, spanning a wide range encompassing the visible, infrared, and ultraviolet regions. The capacity to adjust the properties of the material allows for the production of visual displays with vivid hues, imaging systems with high resolution, and light-emitting diodes (LEDs) that are energy-efficient. Quantum dot LEDs have exhibited enhanced colour fidelity, energy efficiency, and extended operational longevity in contrast to conventional display technologies [17]. Quantum dots demonstrate a notable benefit in terms of their elevated photoluminescence quantum yield [18]. Quantum dots exhibit high efficiency in light emission upon excitation by photons, rendering them suitable for utilisation in biological imaging and sensing applications. The precise and sensitive detection of biomarkers is facilitated by the bright and narrow-band fluorescence signals, which have contributed to advancements in diagnostics, drug discovery, and personalised medicine [19, 20]. Quantum dots have been found to be useful in solid-state lighting due to their high photoluminescence quantum yield, which results in improved energy efficiency and decreased power consumption. Quantum dots exhibit remarkable photostability, indicating their ability to sustain their optical characteristics for extended durations without substantial deterioration [21]. The maintenance of stability is of utmost importance in various applications, including imaging and sensing, wherein precision and uniformity of measurements hold significant value. The utilisation of imaging technologies based on quantum dots provides a means of visualising biological structures in real-time with high resolution [14]. Furthermore, the electronic properties of quantum dots can be altered by doping them with impurities, which presents an opportunity to create transistors and sensors with exceptional efficiency. The distinctive characteristics and adaptability of quantum dots have resulted in a diverse array of practical uses. Quantum dot-based photovoltaic cells present various benefits in the realm of solar energy harvesting, including but not limited to the ability to adjust bandgaps, the capacity for multiple exciton generation, and heightened absorption of sunlight. The aforementioned characteristics facilitate the development of solar cells that are economically viable and highly efficient, capable of converting a wider spectrum of solar radiation into electrical energy [13].

Moreover, quantum dots have exhibited potential in the field of catalysis, facilitating chemical reactions with enhanced efficiency and selectivity [22]. Nanoparticles possess the potential to be customised in terms of their dimensions and chemical makeup to augment their catalytic efficacy, rendering them highly advantageous in domains such as fuel synthesis, elimination of pollutants, and retention of hydrogen [3]. The extensive benefits and potential uses of quantum dots are continually expanding as scholars discover novel prospects. Quantum dots possess a range of advantageous properties, including tunable optical characteristics, high photoluminescence quantum yield, exceptional photostability, and controllable electrical features [5, 8, 23–31]. These attributes render quantum dots a highly valuable resource in various fields, such as optoelectronics, imaging, sensing, energy harvesting, and quantum computing. With ongoing advancements and research, quantum dots are poised to revolutionize various industries and contribute to the development of innovative technologies with significant societal impact [32–34].

The objective of this chapter is to furnish a thorough and all-encompassing exposition on nanocomposites that are based on quantum dots. The emphasis will be on their synthesis, properties, and wide-ranging applications. The distinctive optical and electronic characteristics of quantum dots, which are dependent on their size, in conjunction with the adaptable nature of nanocomposite materials, present promising prospects for progress in diverse domains such as optoelectronics, sensing, catalysis, and biomedical applications. The introductory section of this chapter will provide an overview of the essential principles and characteristics of quantum dots. Emphasis will be placed on their optical behaviour, which is dependent on their size, as well as their remarkable photoluminescence quantum yield and photostability. The forthcoming chapter will explore the various methods utilised for the synthesis of quantum dots, encompassing colloidal synthesis, epitaxial growth, and chemical vapour deposition. The chapter will analyse the benefits, difficulties, and recent advancements associated with each technique. The focus of this study will be on the techniques employed for regulating the dimensions, morphology, and constituent elements of quantum dots, thereby facilitating meticulous adjustment of their optical and electronic characteristics. The final section of the chapter will provide a succinct overview of the present obstacles and forthcoming prospects in the realm of nanocomposites that utilise quantum dots. The objective of this chapter is to furnish the readers with a thorough comprehension of the techniques employed in the synthesis of nanocomposites based on quantum dots, their characteristics, and their potential applications. This is intended to encourage additional research and advancements in this swiftly expanding domain.

2 Chemical Synthesis Techniques

Synthesising quantum dot polymer composites at the colloidal level is a flexible strategy for creating new materials with interesting characteristics and functions. Quantum dots (QDs) are synthesised by incorporating them into a polymer matrix

via a colloidal technique, which allows for nanoscale QD dispersion. The resultant composite materials are promising for use in domains including optoelectronics, sensors, and energy devices due to their improved optical, electrical, and mechanical capabilities. QDs synthesis is the initial stage of colloidal synthesis. Nanocrystal semiconductors such as cadmium selenide (CdSe), lead sulphide (PbS), and indium phosphide (InP) are what make up quantum dots. Several chemical techniques, including hot-injection, sol-gel, and microwave-assisted synthesis, are available for synthesising these nanocrystals. The desired qualities and the specific QD material dictate the synthesis process chosen. QDs' optical and electrical characteristics may be fine-tuned by manipulating their size, shape, polarity, and surface chemistry during production. The QDs' surfaces are often changed with organic ligands after synthesis. These ligands are essential for improving the QDs' compatibility and stability in the polymer matrix. The QDs are more dispersed in the polymer matrix because they are surrounded by a protective layer. To improve the polymer's compatibility, ligands are often exchanged for their native counterparts using a ligand exchange method. The quantum dots with their functionalized surfaces are then suspended in an appropriate solvent. Uniform and steady dispersion needs precise regulation of process variables such temperature, solvent, and mixing methods. Sonication, magnetic stirring, or high-shear mixing are only some of the methods used to effectively disperse QDs and avoid their clumping together. The polymer matrix can be made independently of the QD colloidal solution. The composite material's intended use and desirable characteristics will dictate the polymer of choice. Polymers including poly(methyl methacrylate), polystyrene, and polyvinyl alcohol (PVA) are frequently employed. Solution casting, melt blending, and in-situ polymerization are all viable options for synthesising the polymer matrix. The composite material is formed by combining the QD colloidal solution with the polymer matrix by mixing procedures. Getting the appropriate characteristics requires evenly dispersing QDs throughout the polymer matrix. Various mixing methods are used to disperse QDs evenly throughout the polymer matrix, including stirring, ultrasonication, and high-shear mixing. The resultant material is malleable and may be shaped into films, fibres, or whatever else is needed. Films can be formed using a variety of methods, including spin coating, dip coating, and solvent evaporation. Processing parameters may be adjusted to customise the composite's qualities to a variety of uses by influencing its thickness, shape, and orientation. In order to improve the interfacial interactions between the quantum dots and the polymer matrix, post-treatment techniques may be used. The connection between QDs and the polymer matrix, for instance, can be increased by thermal annealing, leading to enhanced mechanical and electrical characteristics. The composite material's overall stability and durability can be increased by the use of crosslinking procedures. Quantum dot synthesis, surface functionalization, colloidal dispersion, polymer matrix preparation, composite creation, and post-treatment are the main processes in the colloidal synthesis of quantum dot polymer composites. To get the required characteristics and functions of the composite material, optimisation of synthesis parameters and processing conditions is essential. The resultant polymer-quantum-dot composites show great promise for use in several areas, including electronics, photonics, sensing, and energy conversion.

3 Sol-Gel Synthesis

The sol-gel method for synthesising quantum dot-polymer composites is a highly innovative and adaptable approach for producing nanocomposites that exhibit exceptional optical and material characteristics. The composites under consideration integrate the distinct attributes of quantum dots (QDs) and polymers, thereby presenting a diverse array of potential applications in various domains including optoelectronics, sensing, photonics, and biomedical engineering. This article aims to examine the fundamental principles underlying sol-gel synthesis and its utilisation in the fabrication of quantum dot-polymer composites.

The sol-gel synthesis technique is a solution-based approach that facilitates the precise development of nanoscale materials by means of the creation of colloidal solutions or sols. The aforementioned procedure entails the hydrolysis and condensation of metal alkoxides or metal chlorides, resulting in the formation of a cohesive network or gel. The sol-gel method offers various significant benefits in the context of QD-polymer composites. These advantages encompass the capacity to finely adjust the dimensions and structure of quantum dots, as well as their distribution throughout the polymer matrix. Consequently, this technique facilitates the augmentation of properties and functionalities in the resulting composites.

The initial stage in the sol-gel synthesis process of QD-polymer composites entails the preparation of the quantum dots. Quantum dots are semiconductor crystals at the nanoscale that demonstrate optoelectronic properties that vary with their size. Quantum dots possess the advantageous capability of being able to finely regulate their emission spectra over a broad spectrum of wavelengths by means of precise control over their size and composition. This characteristic renders them highly suitable for a diverse array of optoelectronic applications.

Frequently employed materials for quantum dots encompass cadmium selenide (CdSe), cadmium sulphide (CdS), lead sulphide (PbS), and indium phosphide (InP). The synthesis of quantum dots typically involves a distinct reaction, frequently conducted in the presence of surfactants, with the aim of impeding aggregation and regulating the size of the particles. After the synthesis process, the quantum dots are subsequently integrated into the sol-gel matrix. In the subsequent stage, the polymer precursor, typically consisting of a metal alkoxide or a silane-based compound, is combined with the quantum dot solution in order to generate a uniform blend. The aforementioned mixture is subsequently exposed to a sol-gel process, encompassing hydrolytic and condensation reactions. During the process of hydrolysis, the metal alkoxide undergoes a chemical reaction with water, resulting in the formation of metal hydroxide species. Following this, the metal hydroxides undergo condensation, resulting in the formation of a three-dimensional network that incorporates the quantum dots within the developing sol-gel matrix.

The sol-gel process provides precise manipulation of the dispersion of quantum dots within the polymer matrix, thereby guaranteeing homogeneous distribution and inhibiting the formation of agglomerates. The attainment of uniformity plays a critical role in the realisation of the desired characteristics and the improvement of the overall

functionality of the composite material. Following the conclusion of the sol-gel process, it is customary to subject the resultant QD-polymer composite to annealing or curing procedures. These treatments serve the purpose of eliminating any residual solvent and enhancing the mechanical characteristics of the material. The annealing process additionally facilitates the promotion of cross-linking within the polymer matrix, leading to heightened levels of stability and durability. The sol-gel synthesis technique for producing QD-polymer composites presents numerous advantages in comparison to alternative conventional methods. One of the key benefits lies in the capacity to manipulate the dimensions and morphology of quantum dots, thereby exerting a direct influence on their optical characteristics. Moreover, the sol-gel process facilitates the integration of a diverse array of polymers, thereby facilitating the customization of the material's mechanical and chemical characteristics to meet specific application demands. The optical properties of the QD-polymer composites are distinct due to the quantum confinement effect of the quantum dots that are embedded within them. The aforementioned phenomenon results in the ability to adjust emission wavelengths and improve light absorption, rendering these composites exceedingly appealing for utilisation in various applications, including light-emitting diodes (LEDs), solar cells, and photodetectors. In addition, QD-polymer composites have exhibited promising capabilities in the field of biomedical applications, specifically in areas such as bioimaging and drug delivery. The adjustable emission properties of quantum dots can be employed for precise imaging of biological tissues and cells, while the polymer matrix offers a biocompatible medium for the encapsulation of drugs and regulated release. In summary, the utilisation of sol-gel synthesis for the production of QD-polymer composites exhibits significant potential in the development of versatile nanomaterials possessing customised characteristics. The precise manipulation of quantum dot dimensions and distribution within the polymer matrix presents novel opportunities for the advancement of optoelectronic devices, sensors, and biomedical tools. As further investigation and enhancement of this synthesis method are undertaken by researchers, it is anticipated that there will be a proliferation of compelling and influential applications of quantum dot-polymer composites in forthcoming times.

A report discussed the synthesis of Cu-In-S quantum dots (QDs) that exhibit a yellow emission and are encapsulated with double ZnS shells. These QDs demonstrate a remarkably high quantum yield of 92%. Additionally, the report covers the preparation of a composite plate consisting of QDs embedded in polymethylmethacrylate, which is free-standing. Furthermore, the application of this QD plate in the fabrication of white-light-emitting diodes (WLEDs) based on quantum dots is explored [35]. The synthesis of a quantum dot-silica monolith (QD-SM) (Fig. 1) material with exceptional luminescence and photostability was achieved through a two-step process involving the initial surface exchange of quantum dots (QDs) followed by base-catalyzed sol-gel condensation of silica [36]. The utilisation of a propylamine catalyst played a crucial role in preserving the initial luminescent properties of the quantum dots (QDs) within the sol-gel condensation process.

Kim presented findings on the production of a film composed of quantum dots (QDs) encapsulated in siloxane (referred to as QD-silox film). This film demonstrated

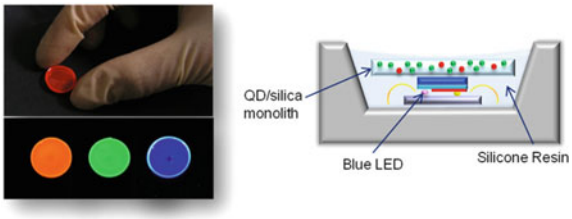

Fig. 1 Fabricated QDs-polymer composites and their graphical illustration of the fabrication. Reproduced with the permission from Ref. [36]© 2013 American chemical society

remarkable stability in terms of emission intensity, maintaining its performance for a duration exceeding one month, even under conditions of elevated temperature and humidity. The QD-silox films undergo solidification through a free radical addition reaction involving oligosiloxane resin and ligand molecules on the QDs [37]. The QD-oligosiloxane resin demonstrates optical clarity even after a storage period of 40 days, which is in stark contrast to alternative resins containing QDs that undergo turbidity and eventually precipitate. This study introduces a novel and straightforward technique for the synthesis of layer-by-layer (LbL) self-assembled films that integrate quantum dots (QDs) and exhibit strong photoluminescence (PL) across a wide spectral range, ranging from blue to red. The utilisation of sol-gel-derived glass layers, which are produced through the hydrolysis of 3-aminopropyltrimethoxysilane (APS) or 3-mercaptopropyltrimethoxysilane (MPS), has been employed as a means of connecting QD layers [38]. Characterization techniques such as absorption, photoluminescence spectroscopy, transmission electron microscopy, and atomic force microscopy were utilised to investigate the properties of the prepared films. The results of these analyses indicated that the QDs in the films exhibited a nearly close-packed coverage and were not found to be aggregated. The photoluminescence (PL) efficiencies of the QDs, specifically CdTe or ZnSe, both stabilised with thioglycolic acid, when dispersed in the films, were approximately 50% lower compared to the PL efficiencies of the initial colloidal solutions. However, the PL efficiencies of the dispersed QDs reached 24% before accounting for a refractive index correction. Phenyl-vinyl-oligosiloxane base resins, which incorporate quantum dots (QDs), were synthesised through an in situ condensation reaction involving sol-gel precursors and QDs. This reaction was carried out both with and without the addition of 3-(mercaptopropyl)trimethoxysilane [39]. Subsequently, the base resins underwent a curing process with the crosslinker resin via hydrosilylation in order to produce phenyl hybrimer (QDPH) resins that incorporate QDs. Our findings indicate that the QDPH resin incorporating a thiol group demonstrated superior transmittance and quantum efficiency compared to the resin without the thiol group. This can be attributed to the more uniform dispersion of quantum dots within the resin. The authors present a straightforward and efficient method for synthesising composites of QDs and polymers. In this study, we incorporated fluorescent semiconducting CdSe/ZnS QDs into various commercially accessible polymers through a straightforward

single-step process [40]. It was observed that the introduction of an excessive amount of free octadecylamine (ODA) into the solution containing QDs and silica led to the formation of a gel. The duration of gelation was observed to be contingent upon the concentration of free ODA. In a similar vein, it was observed that the emission of QD-polymer composites was contingent upon the concentration of free ODA. In a separate study, researchers have successfully synthesised films composed of silica/siloxane embedded with quantum dots (QDs). This was achieved through the process of silica growth and siloxane encapsulation, utilising a straightforward sol-gel reaction [41]. The quantum dots (QDs) were immobilised within an amorphous silica matrix and chemically dispersed within an oligo-siloxane resin (Fig. 2), thereby effectively addressing the issue of aggregation during extended periods of storage. Furthermore, the absence of a solvent in the composite eliminated the requirement for a solidification process. The dual improved S-QD/siloxane film exhibited enhanced long-term performance in challenging conditions such as high temperature (120 °C), high humidity (85% relative humidity), exposure to ethanol, and application of a current bias (100 mA), without the need for an additional encapsulation layer. This performance surpasses that of the previously reported OA-QD/siloxane film.

4 Physical Synthesis Techniques

The utilisation of physical synthesis techniques in the production of QDs-polymer composites presents a distinctive and efficient strategy for the development of nanocomposites with customised properties suitable for a diverse array of applications. The aforementioned methodologies encompass the direct physical amalgamation or integration of quantum dots into polymer matrices, thereby facilitating meticulous regulation of the composite's configuration, dimensions, and quantum dot dispersion. This article aims to examine several prominent physical synthesis techniques employed for the production of quantum dot-polymer composites, and to elucidate their importance across diverse domains.

Solution mixing is a commonly employed and straightforward technique for the preparation of composites consisting of QDs and polymers. The present methodology involves the combination of pre-synthesized quantum dots and a polymer solution, resulting in the formation of a uniform mixture. Subsequently, the amalgamation is applied onto a substrate through either casting or spin-coating techniques, thereby resulting in the formation of the composite film. The utilisation of solution mixing facilitates the seamless integration of diverse quantum dots and polymers, thereby facilitating the customization of composite characteristics to cater to specific applications, including but not limited to optoelectronics, sensors, and bioimaging.

The process of in situ polymerization entails the concurrent formation of polymer chains and quantum dots within a shared reaction medium. The utilisation of this methodology facilitates the seamless incorporation of quantum dots into the polymer matrix during the polymerization procedure, leading to a significant enhancement in compatibility and enhanced dispersion of QDs within the composite material.

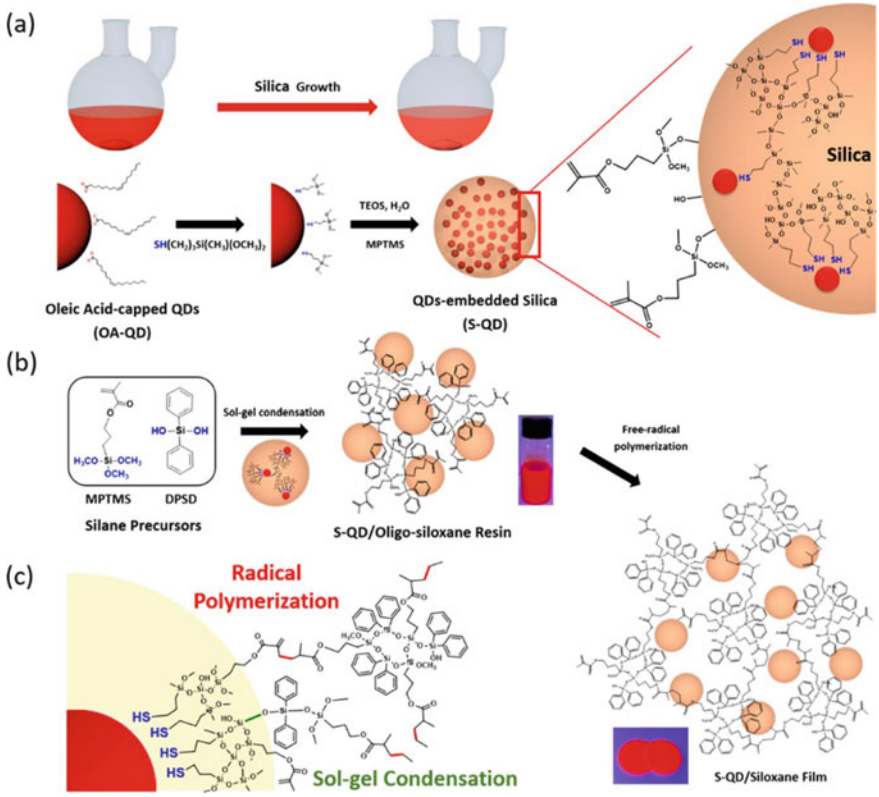

Fig. 2 Design concept of an S-QD/siloxane film. **a** Synthesis process and chemical structure of S-QD. **b** Sol–gel condensation reaction between MPTMS, DPSD, and S-QD. **c** Fabrication of an S-QD/siloxane film by UV-initiated free-radical polymerization between methacrylate groups of S-QD and oligo-siloxane resin. S-QD is chemically bonded to oligo-siloxane resin by radical polymerization and sol–gel condensation. Inset photographs are S-QD/oligo-siloxane resin and S-QD/siloxane film under 365 nm UV light. Reproduced with the permission from Ref. [41]© 2019 American chemical society

The utilisation of in situ polymerization proves to be highly advantageous in cases where monomers possess functional groups capable of interacting with the surface of quantum dots, thereby augmenting the overall stability and properties of the composite material.

Electrospinning is a highly adaptable and scalable methodology employed for the production of nanofibers or non-woven mats through the utilisation of polymer solutions. The production of QD-polymer nanofibers or mats can be achieved by introducing quantum dots into the polymer solution prior to the electrospinning process. The aforementioned technique presents a notable surface area-to-volume ratio and distinctive fibre morphology, rendering it appealing for implementation in various fields such as tissue engineering, sensors, and flexible electronics.

The technique of layer-by-layer (LBL) assembly is a method of controlled deposition that entails the sequential adsorption of species with opposite charges, such as polyelectrolytes and quantum dots, onto a substrate. The construction of multilayered quantum dot-polymer films is achieved by employing alternating deposition techniques, which enable meticulous manipulation of the quantum dot layer count and polymer thickness. The utilisation of LBL assembly presents the opportunity to manipulate optical properties and create customised multilayered structures with specific functionalities.

Emulsion polymerization is a method employed for the production of polymer particles characterised by well-regulated dimensions and minimal size variations. The formation of QD-polymer nanocomposite particles can be achieved by introducing quantum dots into the monomer droplets during the process of emulsion polymerization. The aforementioned particles have the potential to undergo additional processing in order to produce composite films or coatings that exhibit improved optical and mechanical characteristics.

The process of melt mixing entails the direct dispersion of quantum dots into a molten polymer through the utilisation of mechanical stirring or twin-screw extruders. This methodology is specifically well-suited for thermoplastic polymers and is frequently employed within the plastics industry. Melt mixing presents an economically viable and scalable approach for the fabrication of quantum dot-polymer composites, rendering it well-suited for the mass production of various items including LEDs, displays, and wearable electronics.

The physical synthesis techniques employed in the production of QDs-polymer composites offer numerous advantages when compared to alternative methods, such as chemical synthesis or in situ QD growth. Initially, the utilisation of pre-synthesized quantum dots in physical synthesis facilitates meticulous regulation of their dimensions, morphology, and chemical makeup. The implementation of this control mechanism plays a crucial role in the adjustment of the optical and electronic characteristics of the resultant composite material. Furthermore, physical synthesis methods possess inherent advantages such as simplicity, scalability, and the absence of intricate reaction conditions, rendering them appealing for industrial utilisation. The utilisation of physical synthesis techniques in the development of QDs-polymer composites presents a flexible and efficient approach for the fabrication of nanocomposite materials with customised characteristics. Various methods, such as solution mixing, in situ polymerization, electrospinning, and melt mixing, offer distinct advantages that are well-suited for specific applications in the fields of optoelectronics, photonics, sensors, and biomedical engineering. As scholars persist in investigating and enhancing these methodologies, the utilisation of QDs-polymer composites exhibits considerable potential in propelling numerous technologies and fostering innovation across a range of industries.

5 Vapor Deposition

Vapour deposition is a highly effective and extensively employed physical synthesis technique utilised for the production of QDs-polymer composites, offering meticulous regulation over the deposition process. The procedure entails the deposition of vaporised quantum dot materials onto a substrate, succeeded by the integration of the deposited quantum dots into a polymer matrix. The utilisation of vapour deposition presents a multitude of benefits in the fabrication process of quantum dot-polymer composites. This technique allows for improved regulation of quantum dot dimensions, concentration, and spatial arrangement within the polymer matrix. Consequently, this level of control facilitates the customization of material characteristics to suit diverse applications. The principles underlying vapour deposition are based on the process of vaporising quantum dot precursors. This vaporisation can be accomplished using different techniques, including thermal evaporation, sputter deposition, chemical vapour deposition (CVD), or electron beam deposition. Thermal evaporation involves the application of heat to the quantum dot precursors contained within a crucible, resulting in their evaporation and subsequent formation of a vapour plume. Subsequently, the quantum dots in a vaporised state are guided towards the substrate, where they undergo condensation and subsequently adhere as a thin film. The process of sputter deposition entails subjecting a target comprising quantum dot material to the impact of high-energy ions, resulting in the expulsion of atoms and their subsequent deposition onto the substrate. CVD, in contrast, employs reactive gases to produce quantum dot precursors in the vapour phase. These precursors subsequently undergo reactions with the substrate or other gases, resulting in the direct formation of quantum dots on the surface of the substrate. Electron beam deposition is a technique that employs an electron beam to vaporise the quantum dot material, thereby enabling meticulous regulation of the evaporation rate and facilitating the deposition of intricate nanostructures. The precise control over the growth of quantum dots is a significant advantage provided by vapour deposition. The manipulation of deposition conditions, including temperature, pressure, and precursor concentration, by researchers enables the attainment of a specific quantum dot size. This process leads to the uniform distribution of quantum dot sizes and the controlled density of quantum dots within the polymer matrix. The ability to finely adjust the optical, electronic, and mechanical characteristics of the composite material is crucial, rendering it exceedingly appealing for a wide range of applications in the fields of optoelectronics, photonics, and sensors. Furthermore, the utilisation of vapour deposition techniques results in enhanced dispersion and uniformity of quantum dots within the polymer matrix, surpassing certain solution-based methodologies. The direct deposition of quantum dots onto the substrate surface mitigates the likelihood of clustering and agglomeration, resulting in heightened stability and enhanced material performance. Achieving a uniform dispersion of quantum dots throughout the polymer matrix is of utmost importance in order to enhance the optical and electronic characteristics of the composite. Consequently, vapour deposition has emerged as a favoured technique for fabricating QD-polymer composites with superior quality. In addition, vapour

deposition demonstrates compatibility with a diverse array of polymer materials, encompassing thermoplastics, thermosets, and elastomers. The inherent flexibility of composites enables researchers to customise their properties to suit particular applications, including but not limited to flexible electronics, light-emitting devices, and nanoscale sensors. The capacity to fabricate composite materials using a diverse range of polymers facilitates the advancement of multifunctional materials that are well-suited for a wide array of industry requirements. It can be stated that vapour deposition is an extensively adaptable and manageable physical synthesis method employed for the production of quantum dot-polymer composites. Vapour deposition facilitates the customization of material properties for diverse applications in optoelectronics, photonics, sensors, and related fields by providing precise manipulation of quantum dot size, density, and distribution within the polymer matrix. This level of control over nanocomposite properties is crucial for achieving optimal performance and functionality. The ongoing investigation and enhancement of this methodology possess significant potential for propelling nanotechnology forward and fostering innovation in diverse sectors. New heterogeneous CVD polymerization techniques are based on conventional wet-chemical chain- and step-growth mechanisms. Because of their similarities to inorganic CVD techniques, hybrid device fabrication is simplified. CVD polymers unite the fields of microfabrication with the building of complex systems of chemical, biological, and nanoparticles [42]. Covalent grafting can create robust interfaces, allowing for high-resolution (60 nm) patterning on flexible substrates. CVD polymerization is suitable for thermally sensitive substrates like paper, textiles, and plastics due to its use of low-energy input to drive selective chemistry, minimal vacuum, and room-temperature substrates. The potential for lowering environmental, health, and safety implications associated with solvents makes CVD approaches attractive for the production of insoluble and infusible films made from materials including fluoropolymers, electrically conductive polymers, and controllably crosslinked networks. High biocompatibility and low cellular toxicity composite material consisting of polymer coated quantum dots (QDs) and carbon nanotubes was synthesised, as reported by Balsak et al. X-ray photoelectron spectroscopy, laser scanning confocal microscopy, transmission electron microscopy, and scanning electron microscopy were used to characterise the MWCNT-QD-(-poly(glycidyl methacrylate)) (pGMA) hybrids that were synthesised [43].

6 Electrochemical Synthesis

Due to its widespread use in fields as varied as optoelectronics, sensing, bioimaging, and energy storage, the production of quantum dots (QDs) and their integration into polymer matrices has attracted a lot of attention in recent years. Electrochemical synthesis is a versatile and ecologically friendly process for creating QDs-polymer composites with perfect control over the size, shape, and composition of quantum dots, making it a standout among the numerous synthesis methods. This article delves

into the benefits and possible uses of the electrochemical synthesis process for QDs-polymer composites. The production of quantum dots in a polymer matrix can be electrochemically synthesised by driving redox processes with an electric current. An electrolytic cell with a working electrode, a counter electrode, and an electrolyte solution is commonly used for the procedure. In most cases, indium tin oxide (ITO) is used for the working electrode, while platinum or graphite is used for the counter electrode. The QDs' building blocks—metal ions and ligands—are already present in the electrolyte solution. There are two primary methods for the electrochemical synthesis of QDs-polymer composites: in situ synthesis and ex situ synthesis. The polymer matrix is directly involved in the electrochemical reaction during in situ synthesis, resulting to the creation of QDs and polymer at the same time. Benefits of this method include increased homogeneity, precise regulation of QD sizing and distribution, and robust QD–polymer matrix interactions. In order to synthesise anything where it will ultimately be employed, an electrochemical reaction is performed with a monomer that has functional groups capable of interacting with the metal ions. The QDs within the expanding polymer chain are formed when metal ions are produced during the redox process and coordinate with the monomers. Separate production of the quantum dots is followed by their incorporation into a pre-formed polymer matrix using electrochemical methods in ex situ electrochemical synthesis. In this method, the desired quantum dot qualities can be synthesised in advance of their incorporation into the polymer, providing greater control over the final product. Applying a potential to the polymer matrix during the ex situ synthesis helps the QDs migrate from the solution to the polymer surface, where they can be incorporated. There are a number of benefits to using electrochemical synthesis to create QDs-polymer composites instead of more traditional chemical approaches. To begin, the procedure is straightforward, economical, and extensible, thus it may be used for mass manufacture of QDs-polymer composites. Second, the size, shape, and composition of quantum dots may be precisely controlled through electrochemical production, providing greater scope for tuning their properties. The ability to modify the QDs' properties is essential for enhancing their functionality in targeted settings. Thirdly, the environmental impact of electrochemical synthesis is low because it may be carried out in benign settings without the use of harmful reagents. Several different fields have benefited from the electrochemical synthesis of QDs-polymer composites. These composites are used to create high-efficiency LEDs, PV cells, and lasers in the field of optoelectronics. Quantum dots (QDs) are attractive candidates for next-generation electronic devices due to improvements in charge transport characteristics and stability achieved through their incorporation into polymer matrices. In addition, QDs-polymer composites have shown promising results as sensing materials thanks to their sensitivity to a wide range of analytes and enhanced selectivity made possible by polymer functionalization. QDs-polymer composites have great brightness, photostability, and biocompatibility, which paves the way for accurate imaging and tailored drug administration in bioimaging and biomedical applications. These composites have also been investigated for potential use in energy storage devices like supercapacitors and rechargeable batteries due to

their high surface area and electrochemical characteristics. QDs-polymer composites with customised characteristics can be easily and effectively synthesised using electrochemical synthesis. Electrochemical synthesis of quantum dots is appealing because of its environmental friendliness and the fact that its size, shape, and composition can be controlled. It is anticipated that the expanding use of QDs-polymer composites synthesised using electrochemical methods will further drive innovation in the disciplines of optoelectronics, sensing, bioimaging, and energy storage as this area of study develops.

By simultaneously producing the corresponding precursors using cadmium sacrificial anode electrochemical methods, a unique, flexible, and clean colloidal processing strategy was devised for synthesising CdX (X = Te, Se, or S) quantum dots (QDs) [44]. The 3-mercaptopropionic acid (MPA)-stabilized CdX QDs were synthesised in a room-temperature, one-pot electrochemical technique utilising an aqueous medium in around 16 min. Very stable colloidal quantum dots with uniform narrow-size distributions and an average nanoparticle diameter of around 3.0 nm were created, as evidenced by UV–vis, PL, FTIR, TEM-EDS, XRD, DLS, XPS, and Zeta potential characterizations of the CdX quantum dots conjugates. Poly(vinyl alcohol) (PVA) was used as the matrix polymer and coal-derived graphene quantum dots (GQDs) were used to create luminescent polymer composite materials, which were cast from aqueous solutions (Fig. 3) [45]. Fluorescent characteristics are transferred to the polymer matrix from the coal-derived GQDs, and solid state fluorescence is observed in the resulting composite films. The PVA/GQD nanocomposites' optical, thermal, and fluorescent characteristics have all been investigated. At GQD concentrations between 1 and 5 wt%, the composite films display high optical transparency (78–91%) and good dispersion of the nanoparticles. CdSe quantum dots (QDs) were placed into a polymer matrix to create a film with electrochromic, electrofluorescent, and light-induced colouring properties. The film's excellent electrochromic features include low redox potential, strong optical contrast, and fast response time; these benefits are the result of a combination of those presented by CdSe QDs and those presented by conjugated electrochromic polymers [46]. Since the composite film may change between a non-fluorescent and a fluorescent state, with PL intensity greater than twice that of a pure polymer film, higher performance levels are attained. MoS2 is a promising luminophore, and its insertion into PS nanostructures may alter their luminescence properties [47]. To improve the electroluminescence property of PS nanostructures, the current study adopts electrochemical anodization of nanostructured porous silicon, followed by the integration of MoS2 quantum dots (QDs) by spin coating.

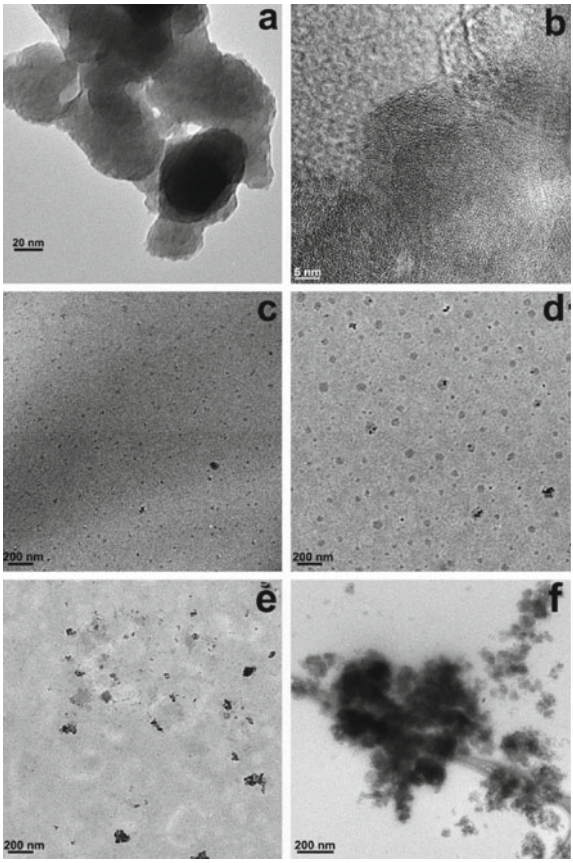

Fig. 3 TEM images of the **a**, **b** GQDs and PVA/GQD composites containing **c** 1 wt %, **d** 3 wt %, **e** 5 wt %, and **f** 10 wt % GQDs. Reproduced with the permission from Ref. [45]© 2015 American chemical society

7 Characterization Techniques for Quantum Dots-Polymer Composites

Nanometer-sized semiconductor quantum dots (QDs) have attracted significant interest for a variety of uses due to their unusual electrical and optical properties. These QDs, when mixed with polymers, create QDs-polymer composites that are superior to both of their constituent parts. The potential of these composites cannot be realised without first adequately characterising them [19]. This article delves into some of the most important characterisation methods used to investigate QDs-polymer composites and learn more about their properties and behaviour.

Spectroscopic methods are indispensable for studying QDs-polymer composites [30, 48, 49]. The optical characteristics of these composites are often analysed using

UV-Vis spectroscopy. It helps scientists evaluate the quantum dots' bandgap, size distribution, and photoluminescence quantum yield by providing data about their absorption and emission spectra [6, 33]. The effect of the polymer on QD luminescence can be deduced by the use of fluorescence spectroscopy, which also aids in analysing the emission behaviour of QDs within the polymer matrix. Spectroscopic methods provide essential information for deciphering the composite's optical characteristics and energy transfer pathways.

Another excellent characterization tool is transmission electron microscopy (TEM), which may be utilised to see the nanoscale shape and distribution of quantum dots within a polymer matrix [50]. By using TEM, scientists may get clear visuals of the QDs and evaluate how they are dispersed throughout the polymer matrix. This method is useful for learning about the size, shape, and distribution of QDs, as well as their propensity to aggregate or cluster [51]. Understanding the charge transfer mechanisms and the overall composite structure is made much easier with the assistance of TEM's evaluation of the interface between the QDs and the polymer.

Quantum dot and polymer matrix crystal structures can be investigated with the use of X-ray Diffraction (XRD) [52]. Quantum dot (QD) and polymer (poly) crystalline phases, lattice parameters, and crystallite size can all be determined from the XRD patterns. The effect of the polymer on the crystal structure can be seen in the diffraction pattern changes, which can be used to improve the composite material [27].

Quantum dot–polymer chemical interactions are typically studied with Fourier–Transform Infrared Spectroscopy (FTIR). Understanding the intermolecular interactions, bonding, and potential alterations to the polymer's structure due to the presence of QDs is made possible with the help of FTIR spectra, which provide information about distinctive functional groups and bond vibrations.

Quantum dots in a polymer matrix can be characterised with great precision using Dynamic Light Scattering (DLS). Insight into particle size distribution, aggregation, and surface charge can be gleaned from DLS measurements of the hydrodynamic size and stability of QDs. This information is crucial for determining the longevity of the QDs in the polymer matrix and for finding the most effective method of dispersing them.

QDs-polymer composites' heat stability and breakdown behaviour are investigated using thermogravimetric analysis (TGA). Researchers can evaluate the impact of the polymer on the thermal characteristics of QDs by analysing the weight loss as a function of temperature, thereby identifying the thermal degradation temperature of the composite. This knowledge is crucial for the development and implementation of these composites in high-temperature settings.

To fully grasp the viscoelastic behaviour of QDs-polymer nanocomposites, rheological investigations are necessary. The ability to optimise processing conditions and forecast the performance of the composite in real-world applications is greatly aided by the information provided by rheological approaches, which shed light on the composite's flow and deformation behaviour. For QDs-polymer composites used in electronics, it is crucial to characterise their electrical and optoelectronic properties. Current-voltage (I-V) measurements, impedance spectroscopy, and charge carrier mobility measurements are all useful for elucidating the composite's electrical

conductivity and charge transport behaviour. When used to devices like solar cells and transistors, these numbers reveal essential information for improving the composites' performance. The ability to accurately characterise QDs-polymer composites is essential for gaining insight into their characteristics and behaviour. Optimising these composites for different uses requires data from a wide range of measures, including spectroscopy, microscopy, thermal, rheological, and electrical. The continued development of these characterisation methods will aid in the progress of QDs-polymer composites, thereby releasing their potential for application in forward-thinking technologies and research.

8 Conclusion

This book chapter has shed light on the synthesis, characterisation, and wide variety of applications of quantum dots-based nanocomposites; a fascinating topic indeed. Quantum dots, with their novel features, have proven to be an invaluable tool in the field of nanotechnology, allowing for significant progress in many other areas. Controlled synthesis of quantum dots, allowing for customization of their size, shape, and composition, has been a recurrent theme throughout this chapter, demonstrating how this factor greatly influences their performance and potential applications. Solar cells have improved light absorption and charge transport efficiency thanks to the inclusion of quantum dots into nanocomposites, leading to remarkable advancements in photovoltaics.

The incorporation of quantum dots in optoelectronic devices has also ushered in a new era of energy-efficient and visually stunning display and lighting systems. Nanocomposites based on quantum dots have shown promising results in a number of biomedical applications, including targeted drug delivery, bioimaging, and theranostics. This chapter has also highlighted the significance of nanocomposites based on quantum dots in environmental applications, highlighting their potential as novel tools for pollution monitoring and environmentally friendly cleanup procedures. One example of their participation in developing eco-friendly solutions is the use of solar energy for environmental remediation. While this chapter has shown the tremendous promise of quantum dot-based nanocomposites, it has also brought to light some difficulties that need to be addressed. There is a pressing need for more research and development in the areas of safety, scalability, and the optimisation of fabrication procedures.

Looking forward, the promise of nanocomposites based on quantum dots is undeniable. Researchers, scientists, and engineers interested in exploring the possibilities of this cutting-edge technology will find this chapter to be an invaluable resource. We can unlock even more useful applications and pave the path for quantum dots-based nanocomposites to become vital instruments in designing a sustainable, efficient, and linked world by tackling problems and expanding on the knowledge offered here.

The ultimate goal of the development of nanocomposites based on quantum dots is to advance nanotechnology into uncharted territory and bring about a better, more promising future.

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Properties of Quantum Dots Based Nanocomposites

Ashish Kumar Bhui, Sanvedna Shukla, Satish Sen, and Amar Dey

Abstract One of the first nanotechnological materials to be combined with nanocomposites as quantum dots (QDs), whose potential for use in a variety of commercial pharmaceutical and medical goods has been greatly anticipated. Within a wide range of swift developing field of material science and technology, these are some of the crucial scientific developments of the current era. Due to their exceptional physicochemical features, quantum dot-based nanocomposites have received a lot of attention recently. Furthermore, at this extent, not only the quantum confinement but also the surface effects become crucial, making it possible to manipulate the quantum dot's surface or diameter so that its various characteristics can be easily explored. Quantum confinement has an impact on the dot's photon emission and absorption. These features have been used in a variety of analytical tests, including electrochemical sensing platforms, because of their exceptional properties paired with their simplicity in synthesis and combination with nanocomposite materials.

Keywords Quantum dots · Nano composites · Quantum confinement · Electrochemical sensing platform

Abbreviations

QDs	Quantum dots
DNA	Deoxyribonucleic acid

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GQDs	Graphene Quantum dots
CT	Charge transfer
TDDFT	Time dependent density functional theory
TPP GQD	Tetraphenylporphyrin Graphne Quantum dots
DSSC	Dye-sensitized solar cell
ESP	Surface's electrostatic potential
NiTPP	Ni(II)tetraphenylporphyrin
E/G	Edge type binding
P/G	Graphene basal plane
NGs	Nanographenes
PCM	Polarizable continuum model
HBC	Hexa-peri-hexabenzocoronene
GNR	Graphene nanoribbon
PBE	Perdew-Burke-Ernzerhof exchange-correlation energy functional theory
SCC-DFTB	Self-consistent charge density-functional–based tight-binding theory
DOSs	Densities of states
CQDs	Carbon quantum dots
PL	Photoluminescence
LOD	Limit of detection
ECL	Electrochemiluminescence
L-CQDs	L-Tryptophan capped quantum dots
CuNCs-CNQDs	Copper nanoclusters@nitrogen-doped carbon dots
ZnO QDs	Zinc oxide quantum dots
VOCs	Volatile organic compounds

1 Introduction

Recent advances in nanomaterials have shown substantial relevance in various areas including optics, informatics, environmental health, biomedicine, electronics energy, and pharmaceuticals [1, 2]. Due to their potential to overcome cell gaps and enter cells, nanoparticles of a size equivalent to cell organelles or lipid biomolecules, deoxyribonucleic acid (DNA), or proteins have garnered researchers' attention. Nanoparticle application has been widely extended in biosensing, tumor cell imaging, and cancer treatment after being coupled with biomolecular affinity ligands prime among them are gold, silver, or iron oxide nanoparticles [3–6]. The semiconductor nanocrystal quantum dot (QD) is extensively probed [7–9]. The Review chapter exclusively deals with various quantum dots in terms of preparation and properties used in the modern context.

2 Quantum Dots and Nanocomposites

Through quantum confinement effect semiconductor nanoparticles as quantum dots (QDs) encompass unique optical and electrical properties. For several sectors, including nanotechnology, electronics, and photonics, they have sparked a great deal of interest. Nanocomposites, which are substances made of a mixture of many types of nanoparticles distributed in a matrix material, can include quantum dots. Quantum dot-containing nanocomposites have several benefits and possible uses. Apart from having a size in the ultra range, wavelength at broad excitation level, superior photostability and optimised size-tuneable-fluorescence, they have been studied as a substitute or supplement to organic dyes [10]. Newly developed QDs possessing properties of near-infrared emission can monitor animals in vivo without radioactive reagents [11]. Pristine QD's nanotoxicity and restricted functionalities limit its biological potential. QD's biocompatibility and roles beyond fluorescent labeling may be improved by combining it with nanomaterials and proteins. QD might be biocompatible and have other uses by combining it with nanomaterials and proteins. Heavy metal leaching can be prevented by coating QD with biocompatible materials without disturbing its optical properties. Nanocomposites that incorporate quantum dots (QDs) are materials that merge the distinctive characteristics of quantum dots with those of matrix material. The term "quantum dots" refers to nanocrystals made of semiconducting materials that exhibit unique opto-electronic features based on size. are The phenomenon of quantum confinement is often considered responsible for all these traits which in turn are based on emission features that are size adjustable, better photoluminescent quantum yield and broad absorption spectra. The incorporation of quantum dots into a matrix material, such as polymers, ceramics, or metals, results in the formation of nanocomposites. These nanocomposites can display unique or improved characteristics when compared to the individual components. Nanocomposites that utilize quantum dots possess several noteworthy characteristics and practical uses. Quantum dots are distinguished by their optical features, which include their high luminescence efficiency and controllable color-changing light emission. These emission features can be harnessed in various applications of optoelectronics, display technologies, and light-emitting devices when integrated into a matrix. The electrical behavior of quantum dots is distinct due to quantum confinement effect. Nanocomposites can be utilized in various applications including energy storage, electronics and sensing due to their ability to display improved electrical conductivity or customized electronic properties when integrated into a matrix. Using quantum dot-containing nanocomposites might be one way to boost light absorption and charge separation in solar cells. The use of quantum dots with a tunable bandgap is advantageous in capturing a broader range of solar radiation. The matrix material serves to provide structural support and facilitate electron transport pathways. The use of quantum dot-based nanocomposites as catalysts is effective due to considerable surface area, soaring surface reactivity and capacity to regulate charge transfer processes. These are used in various fields including photocatalysis, water

splitting, and chemical synthesis. Nanoparticles possess distinctive optical characteristics and surface chemistry that render them highly desirable for variety of functions such as fluorescent labeling, molecular sensing, and targeted therapy in biological systems. Thus nanocomposites incorporating quantum dots (QDs) signify a category of hybrid materials that amalgamate the distinctive characteristics of both QDs and a matrix material. Quantum dots, sometimes referred to as semiconductor nanocrystals, display varying optical and electrical characteristics based on their size. These properties are frequently attributed to the phenomenon of quantum confinement. The aforementioned properties demonstrated by the subject under discussion include emission that can be adjusted according to size, a photoluminescence quantum yield that is notably high, and absorption spectra that are broad as shown in Fig. 1. Integration of quantum dots into matrix material, like polymers, ceramics, or metals, results in the formation of nanocomposites that may display superior or unique characteristics in comparison to the individual constituents. Nanocomposites that incorporate quantum dots exhibit several noteworthy characteristics and have a variety of potential uses. Quantum dots exhibit remarkable optical properties, characterized by their high luminescence efficiency and tunable emission of light across a range of colors, which is attributed to their size-dependent behavior. The integration of these emission characteristics within a matrix can be harnessed for implementation in optoelectronics, display methodologies, and devices that emit light. The integration of nanocomposites into a matrix can result in improved electrical conductivity or customized electronic characteristics. The utilization of nanocomposites containing quantum dots has been proposed as a means of improvising light absorption features along with charge separation in photovoltaic systems. The utilization of quantum dots possessing a modifiable bandgap has the potential to capture a broader spectrum of solar radiation. The matrix material serves the dual purpose of providing structural reinforcement and facilitating electron transport pathways. Biomedical research on quantum dot nanocomposites has been conducted extensively, especially in the domains of drug delivery, biosensing, and biomedical imaging. These substances are utilized in various fields including chemical production, water electrolysis and photocatalysis. Quantum dot nanocomposites has been materialized proficiently in recent times wide areas of biomedicine, particularly in biomedical imaging, drug delivery, and biosensing. Owing to their distinctive optical characteristics and surface chemistry, they are highly enviable for application in varied biological system tasks such as targeted therapy, molecular sensing, and fluorescent labeling. It is noteworthy that the distinct characteristics and utilisations of nanocomposites that rely on quantum dots are subject to variability contingent upon the matrix material implemented, dimensions and constitution of the quantum dots and the methods of fabrication used. Continued investigation in this area endeavours to further examine and utilize the capabilities of these nanocomposites for a diverse array of technological implementations.

Some researched quantum dots based nanocomposites with properties and applications are listed as follows.

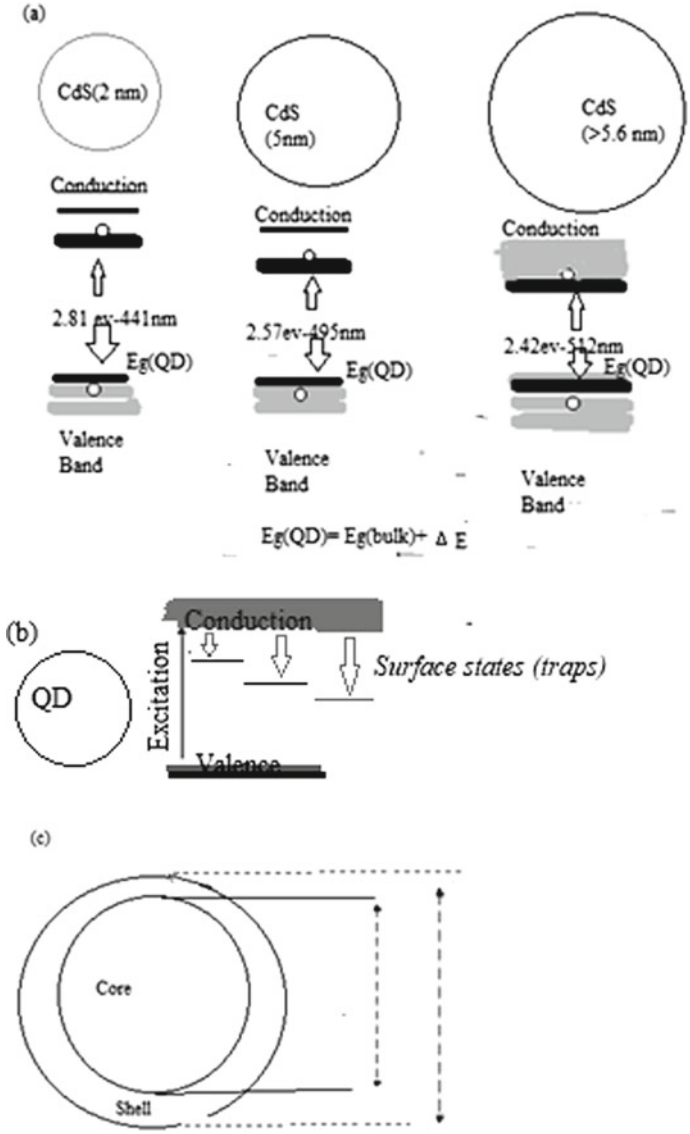


Fig. 1 A diagrammatic representation of different solid band structures **a** quantum confinement effect due to variation in size of quantum dots **b** electronic energy states of QDs bandgaps along with surface trap sites **c** The electronic view showing heterojunction formed by two semiconductor [12]

3 Graphene Quantum Dots (GQDs) Based Nanocomposites

Graphene Quantum dots (GQDs) are class of carbon nanomaterial with many diversified features and high degree of adaptablility. The remarkable qualities of carbon nanoparticles (NPs) are caused by the peculiarities of quantum confinement and edge effects that set them apart from other versatile NPs. GQDs possess exceptional properties such as remarkable biocompatibility, minimal toxicity, water dispersibility, high optical absorbance capability, elevated fluorescence effect and substantial degree of photo stability. Due to their unique qualities, GQDs have acquired their potential applications in delivery, photocatalysis, photovoltaics, nanosensor manufacturing, and photodynamic therapy. A variety of nanocomposites and nanohybrids based on GQDs have been produced and/or analysed through computational means.

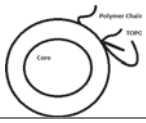
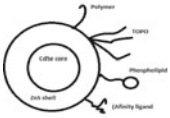
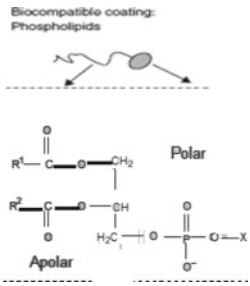
GQDs are zero-dimensional materials with nanoparticle sizes < 10 nm [13]. GQDs are distinct from carbon dots due to the fact that they possess less than 10 carbon atom layers, thickness below 100 nm and contain graphene lattice [14]. GQDs, carbon nanotubes (CNTs), fullerenes, graphene, graphene oxide, and carbon dots (CDs) are all carbon nanomaterials. GQDs are distinguished by edge effects and quantum confinement in their graphene-like cores. GQDs have a limited, controllable band gap like semiconductor QDs [15]. GQDs may be synthesised in huge quantities from inexpensive and plentiful carbon sources without using expensive and ecologically damaging heavy metals [16]. GQDs are promising candidates for sensors [17–22], imaging [23–25], photovoltaics [26–28] and displays [29], energy production [30–34], medicine [13, 25, 35–42], and catalysis [43, 44], Fernando et al. [45, 46]. GQDs are hydrophobic and less poisonous than graphene [47]. In terms of biocompatibility, aqueous medium dispersibility, optical absorption, fluorescence activity, and photostability GQDs do better than typical semiconductor QDs and other carbon-based NPs [47–51]. GQDs create versatile nanocomposites with various chemicals [37, 51, 52].

4 Porphyrin-Quantum Dots-Nanocomposites and Their Derivatives

Porphyrins are promising dye-sensitized solar cell (DSSC) sensitizer owing to their easily mutable chemical structures apart from other features like ample absorption capacity in the near UV–vis range and fair degree of photostability [53]. Compared to other sensitizers Porphyrins absorption is weaker in solar light energy range of 400–600 nm [18, 54–56]. Thus these are good solar energy harvesting sensitizer which absorbs UV to infrared light. Thus, adjusting porphyrin optical absorption improves the efficiency of dye-sensitized solar cells (DSSC). This can be done by different ligands exchange to porphyrins with or by producing nanocomposites/nanohybrides/nanoconjugates. Due to their greater light absorption, dye sensitizer-quantum dot nanocomposites harvested solar energy more efficiently than their

separate components (e.g. [49]). Thus, the amount of solar light absorption can be increased by nanocomposites containing GQDs and porphyrins or their derivatives. Relative enhancement in absorbance was studied through Computation method for tetraphenylporphyrin (TPP)-GQD nanocomposites as solar cell sensitizers to improve DSSC efficiency by observing optical absorbance of diverse GQD/porphyrin ratios 1:1,1:2,1,3, 1:4 and TPP:3GQDs ratio [57]. Based on Li et al.'s extensive explanation of edge-functionalized GQDs, the cis-coronene $C_{54}H_{18}$ was used as a model. The model involved One TPP phenyl binded by amide linkage with GQD, and the rest involved TPP functionalizion at their para-positions. Dispersion adjustments were needed to understand TPP-GQD interactions, The Gaussian 09 B3LYP/Lanl2dz technique optimised the gas phase structures of GQD, TPP, GQD-TPPs, and TPP-GQDs. Harmonic frequencies were computed to test optimised structural stability. Table 1 depicts the step wise synthesis of GQD.

Table 1 Typical process used to produce QDs for bioapplications [68]

Steps of process	Process
(i) Coreshell QDs (CdSe–ZnS	CdSe (Core) with ZnS (Shell) (*Size 2–10 nm*) → capping with capping ligand TOPO (Triooctylphosphine Oxide)
(ii) Organic colloidal stabilization [ligand tri-*n*-octylphosphine oxide (TOPO)];	Capped core → Incepton of PEG (polyethynene glycol) [HO-(CH_2-CH_2-)nCH_2-OH] polymer chain and TOPO polymer chain to surface of core
(iii) Hydrophilic polymer attachment (PEG	[((CdSe $_{core}$)ZnS($_{Shell}$))TOPO($_{capping\ ligand}$)]PEG$_{polymer}$ 
(iv) Bioconjugated with affinity Ligands as targeting molecule (immunoglobulin-G)	Bioconjugated with affinity Ligands as targeting molecule (immunoglobulin-G)
(v) Formation of integrated hybrid biocompatible nanocomposite (micellar structure)	 

Optical absorption characteristics of optimised GQD, TPP, and nanocomposites geometries were studied through Octopus coded computation of TDDFT (Time dependent density functional theory) having for higher GQD-TPPs and TPP-GQD nanocomposites using the 'local-density approximation' and 'Hartwigsen-Goedecker-Hutter pseudopotential' [58]. All structures absorbed infrared. TDDFT demonstrated that adding TPPs or GQDs increased optical absorption. QD-rich nanocomposites have better optical absorption. GQD-3TPPs had a somewhat greater absorption than GQD-4TPPs. GQD-TPPs nanocomposites had greater optical absorptions than those having equal QD number. The lowest unoccupied molecular orbital energies (LUMO) of all nanocomposites exceeded the minimum energy of conduction band of TiO_2, meeting the DSSC criteria. 'Spatial charge separation and electron transport parameters' demonstrated sluggish 'electron-hole recombination' for GQD: TPP conjugates maintaining the ratios 1:2, 1:3 and 1:4 respectively. Despite the same electron transfer driving force, these complexes GQD-TPP, TPP-2GQDs, and TPP-3GQDs were estimated to be rapid. Due of its near UV optical absorption, GQD-3TPPs was the most promising nanocomposites for DSSCs. Karachevtsev et al. [59] performed DFT and MD analyses of meso-5,10,15,20-tetraphenyl porphyrin flattening owing to graphene absorption in the same year. Graphene noncovalent functionalization with organic molecules moieties with extended conjugated systems (porphyrins and their derivatives) can tune its electronic properties and expand its nanotechnology applications [60, 61]. Several research groups computationally examined naphthalene, pyrene, benzene, and other flat-conjugated aromatic hydrocarbon adsorption [43, 62, 63]. These compounds were shown to attach through planar stack conformations to graphene, leading to rise in interaction energies with contact area. Noncovalently bonded molecules and graphene did not transfer charge (CT).

Noncovalently bonded species weakly altered graphene's electrical structure [43, 59, 62, 63]. As predicted, dispersion forces dominated intermolecular interactions in these stacks. Electrostatic interactions contributed significantly less. Graphene functionalization with electron-withdrawing groups and electron-donating groups increased the interaction energies of nanocomposites. The adsorbed molecule's asymmetry also affected BEs. Porphyrins were widely studied in graphene noncovalent functionalization [24, 64–66]. However, basic structural and interaction energy problems remained unresolved [59]. Thus, understanding these systems' interconnections was vital. Further study on effect of porphyrins and meso-5, 10, 15, 20-TPP on graphene was done to determine how adsorption modifies TPP structure [59]. Gaussian 09 involving M05-2X/6-31G(d) was used in to study graphene-P complexes apart from graphene-TPP and their components. BSSE adjustments were also computed [59]. Similar observations was obtained conclusively stating that M05-2X estimated interaction energy without BSSE adjustment matched MP2 interaction energies better. The $C_{216}H_{36}$ fragment, big enough to adsorb the TPP molecule, modelled graphene. MD contained in NAMD package equipped with Charmm27 force field was used to model the production of graphene-P nanohybrids and graphene-TPP. The graphene surface modelling was done using a 120 × 124 Å

graphene sheet rectangular in shape having $C_{5682}H_{212}$ composition. The nanohybrids were placed in water box 148 × 145 × 33 Å size containing 20,000 water molecules. MD simulations frozen graphene atom coordinates. After 500 stages of energy minimization, the simulation ran for 30 ns (1 fs step). Periodic boundary conditions with 303 K temperature and 1 atm pressure were used in simulation. Electrostatic Long-range interactions were computed using Particle Mesh Ewald. PBEs and TPP with graphene have similar DFT energies of −21.5 kcal/mol and −22.6 kcal/mol. Interactions of Porphyrin core-stacking with graphene offered high contribution to its interaction energy. Because the TPP phenyls are outside of the core plane, steric hindrances led the TPP core to approach the graphene surface less than the original porphyrin. The binding energy decrease caused by this impact was countered by the higher van der Waals interactions of the phenyls with graphene, giving TPP or porphyrin the same interaction energies with graphene. The dihedral angle between the core plane and the phenyl planes decreases from 64 to 50° as a result of TPP interactions with the graphene surface, which twist and deform the phenyls and porphyrin core. Through the creation of a small negative potential zone between the porphyrin molecule and the surface, TPP adsorption altered the electrostatic potential (ESP) of the graphene surface. Simulations of MD confirmed DFT findings that TPP and P were stacked on graphene in aqueous conditions. Porphyrins' in-plane motions on graphene result in low-energy barriers and excellent surface mobility. By combining 2D nanomaterials like graphene with aromatic chemicals like porphyrins, novel nanocomposites were created by taking advantage of the conformational behaviour of non-planar molecules on graphene. Graphene and Ni(II)-tetraphenylporphyrin (NiTPP) were covalently bonded by Hou et al. [67] to form a graphene-porphyrin nanohybrid.

They investigated the graphene reactivity of a novel ß-NiTPP-N2+ (the diazonium group connected to the ß-pyrrolic position of the porphyrin core) and meso-NiTPP-N2+ (the diazonium in 4-position of a peripheral phenyl). Due to steric restrictions, the second species could not attach to graphene's surface, only functionalizing GQD edge dangling bonds. The structural characteristics of the graphene-NiTPP hybrid were discovered by structural optimisation at the PM3 level for two model species: Covalent bonding between ß-NiTPP and the graphene moiety edge allows for a center-to-edge joining. A strain-free structure was formed by the edge type binding (E/G-NiTPP), in which the ß-NiTPP unit was oriented perpendicular to the graphene moiety plane, whereas deformations were caused by the covalent binding to the graphene basal plane (P/G).

A geometrically selective bottom-up synthesis of curved nanographenes (NGs) was described by Fernández-García et al. [45]. Different reaction conditions activated seven-membered ring formation, giving the NG negative curvature and creating two topologically unique curved NGs. Electrochemical, photophysical, Raman, X-ray crystallographic, and computational techniques were used to study structure-property connections for these two novel NGs.

These NGs that are curved and have a fused corannulene unit might represent GQD nanocomposites or models. To determine the type of absorptions seen in the

UV-vis spectra, TDDFT calculations were performed using the B3LYP-D3/def2-SVP-optimized grid for both NGs (the polarizable continuum model (PCM) model accounting for CHCl3 effects). The vertical excitations of these compounds were at calc = 479 and 471 nm, or 2.59 and 2.63 eV. These results were excellent with the experimental optical gap values (from excited state measurements): 2.61 and 2.59 eV, and moderately good with the experimental data, exp = 456 (2.72 eV) and 441 nm (2.81 eV). Electron transport from the HOMO (dominated by the polyaromatic hydrocarbon (PAH) domain) to the LUMO (a *-MO fully delocalized over the structure) was a feature of both vertical transitions. The -* transition in the 450 nm absorption was indicative of the prolonged -conjugation of the novel compounds. According to Umeyama et al. [56], these NGs' optical gap values are comparable to those of the reference compound, hexa-tert-butyl-hexa-peri-hexabenzocoronene. The study of graphene nanoribbons (NGs) with sp^2-carbon networks of varying sizes, notably hexa-peri-hexabenzocoronene (HBC) and its rectangularly-extended variant, a short graphene nanoribbon (GNR), covalently functionalized by photoactive Zn-porphyrins, has been recently described, both theoretically and experimentally. 42 sp^2-hybridized carbon HBCs are the smallest NG unit. Because of their distinct zones of -conjugation, graphenes, graphene oxides, chemically transformed graphenes, and GQDs are structurally heterogeneous at the molecular level.

Due to their various regions of conjugation, edge structures, and defects, graphenes, graphene oxides, chemically converted graphenes, and GQDs are structurally heterogeneous at the molecular level (Allen et al. 2010) [69–72].Well-defined extended HBC derivatives provide excellent GQD models, and they may be used to investigate the structure-property correlations of the sp2-carbon species mentioned above [56, 72, 73]. Spectroscopic analysis revealed that the photodynamic properties of porphyrin-linked NGs are strongly influenced by the size of the NG unit [56]. Exclusive energy transfer (EnT) from the first singlet excited state (S1) of the NG to the porphyrin unit was brought about by photoexcitation of the porphyrin–HBC system. The first and second singlet excited states (S1 and S2) of the porphyrin unit displayed opposing selective EnT from the porphyrin–GNR system to the NG. It was demonstrated that the corresponding ultrafast EnTs from the carotenoids S2 and S1 states to chlorophylls in naturally existing photosynthetic light-harvesting systems were replicated by the ultrafast efficient EnTs from the porphyrin S2 and S1 states to GNR. These characteristics make the nanocomposites suitable for solar energy conversion devices and optoelectronics. B3LYP/6-31G* spin-restricted calculations were carried out. Every structure was optimised without any constraints related to symmetry. DFT calculations revealed that the HBC moiety in ZnP–HBC complexes had a larger band gap than ZnP. The ZnP units dominated the ZnP–HBC HOMOs and LUMOs. In contrast to the ZnP–HBC systems, the HOMO and LUMO of the ZnP–GNR were located on the GNR moiety.

Theoretical research on porphyrin-sensitized GQD solar cells' feasibility are also noteworthy [74]. Functionalizing GQDs with porphyrins altered their electrical energy levels, therefore the authors investigated. GQD-porphyrin nanocomposites'

electrical structure was investigated through the use of self-consistent charge density-functional–based tight-binding (SCC-DFTB). Slater-type orbitals and the Perdew-Burke-Ernzerhof (PBE) exchange-correlation energy functional were employed as basis sets. Here's how GQDs were modelled. A huge graphene sheet was created first. (ii) A tiny graphene QD was selected and sliced. (iii) Maintaining symmetry created bigger QDs. GQDs with symmetry up to 10 nm were constructed this manner. GQD-porphyrin nanocomposites were modelled by attaching porphyrin molecules to GQD through amide linkage. Functional groups at phenyl parapositions were employed in metal-free TPP molecules. Hexagonal and trigonal GQDs containing three TPP molecules were examined. The DFTB+code's SCC-DFTB technique calculated these GQD-TPP nanocomposites' total energy. To avoid erroneous interactions with nanocomposite periodic replicas, huge supercells with extensive vacuum regions in all directions were employed. In the Slater-Kirkwood model, dispersion interactions were added. Calculations of absorption spectra using the system containing a single TPP molecule. Nanocomposites can have type-I or type-II band energy alignment, depending on the size of GQDs. Smaller GQD nanocomposites exhibited type-II band energy alignment, where the GQD contributed to the LUMO and the TPP dominated the composite system HOMO. Systems with higher GQDs, however, aligned the type-I band energy. Systems for aligning type-II bands showed evidence of charge separation. Charge carrier recombination was modest in these systems. Smaller GQD nanocomposites were thought to offer greater photovoltaic properties. Porphyrin-GQD nanocomposites with bigger GQDs and electron-donating groups $eOCH_3$ and eNH_2 were also studied. Type-II alignment was identified in the nanocomposites. An extensive analysis of densities of states (DOSs) for nanocomposite materials revealed that the size of GQDs increased the energy gap between the LUMOs of porphyrin and GQD (a measure of electron injection efficiency from photoexcited porphyrin to GQD). Consequently, porphyrin-to-GQD electron transmission is enhanced by GQD size.

The intriguing physico-chemical properties of carbon-based quantum dots and nanocomposites, which are produced via edge effects and quantum confinement, have drawn attention to their potential as sensors. Top-down and bottom-up methods for CQDs, GQDs, and their nanocomposites include laser ablation, arc-discharge, chemical oxidation, ultrasonication, oxidative cleavage, microwave synthesis, thermal decomposition, solvothermal or hydrothermal method, stepwise organic synthesis, carbonisation from small molecules or polymers, and impregnation. Carbon-based compounds are extensively utilised for environmental cleaning because of their large specific surface area, adaptable structure, low toxicity, and superior stability. Semiconductor nanoparticles (NPs) with quantized energy states that are smaller than 10 nm are known as quantum dots (QDs) [75, 76]. More atoms are present on QD surfaces than in bulk materials because of 3-dimensional truncation. According to Reshma et al. [77], Hu et al. [78], QDs have good luminescent and electrical characteristics, such as narrow emission, broad and continuous absorption spectrum, and light stability. QDs based on semiconductors are durable inorganic fluorescence probes. In terms of biocompatibility, chemical inertness, and low cytotoxicity, CQDs and GQDs outperform semiconductor QDs such as CdSe dots, ZnO dots, and others

[79, 80], Alaghmandfard et al. [81]. They show simple synthesis, bioconjugation, and wavelength-dependent luminescence emission [82, 83]. Band gaps that are not zero improve optical characteristics. On their surfaces, functional groups produce edge effects. Wide photoluminescence emission, significant excitation wavelength dependency, and broad absorption bands are characteristics of carbon-based QDs [84]. The majority of researchers [85–87], (Zhang et al. 2019) have employed them in electrocatalysis. Light-emitting LEDs [80], cell labelling [88], cancer imaging and therapy [89], and cell imaging [90].

5 A Comparison Between Graphene and Carbon Quantum Dots (CQDS/GQDS)

With exceptional conductivity, chemical stability, environmental friendliness, high photostability, broadband optical absorption, low toxicity, photobleaching resistance, large surface area, and simplicity of modification, carbon nanoparticles (CQDs) smaller than 10 nm are a remarkable material. The most notable feature of CQDs is their photoluminescence [91]. After prolonged stimulation, CQDs' fluorescence emission intensity remains consistent due to their excellent fluorescence stability. Because of their special qualities, CQDs are perfect for sensing applications [92]. Because of their many carboxyl groups, CQDs can incorporate chemically reactive groups for functionalization or connect with other polymeric, organic, biological, inorganic, or natural components for surface modification. According to Ying Lim et al. [93], surface functionalization promotes solubility in both aqueous and non-aqueous environments, whereas surface passivation improves fluorescence. Because it creates a new energy state inside the band gap, high oxygen levels cause a change in the width of CQDs [94].

GQDs are small graphene particles with lateral dimensions of less than 100 nm, forming two-dimensional nanocrystals. Oxygen, hydrogen, and numerous layers under 10 nm are also present. The band gap energy of GQDs may be adjusted between 0 and 6 eV. Modifying the lateral dimension or surface chemical properties can tune the band gap because of conjugated edge effects and the quantum confinement effect [95]. Because of their superior optoelectronic qualities, affordability of production, and biocompatibility, GQDs can take the position of quantum dots based on metal chalcogenides. Graphene has better thermal and electrical conductivity than semiconductor quantum dots because of its bonding both above and below the atomic plane [96–98]. Among the numerous great qualities of GQDs are tunable photoluminescence (PL), extraordinary spin property, exceptional UV-blocking capabilities, and high photo stability [99]. Redshifting from 522 to 575 nm, the pH of the GQDs solution rises from 1.0 to 11.0 [100]. This results from the deprotonation of functional groups that include oxygen, such as carboxyl, hydroxyl, and epoxy. Because red florescence has a peak at 626 nm, it happens at pH levels higher than 11.0. Being

pH and temperature sensitive, and even a minor alteration can cause a fast fluorescence shift. Biomedical and bioanalytical applications of CQDs and GQDs are growing due to their photoluminescence and ability to interact with biomolecules. CQDs and GQDs' unique optical features might help create sensitive bioanalysis systems for point-of-care diagnostics. Several forms of carbon dots for medicinal use may improve their use. In point-of-care viral testing, CQDs-based biomacromolecule detection has shown great performance and feasibility [101]. Li et al. [102] detected SARS-CoV-2 using Gd^{3+} doped CQDs and specific antibodies. GQDs, gold nanoclusters coupled with antibodies, were used by Yuan et al. [103] to detect infections.

On comparing both CQDs and GQDs it is observed that CQDs and GQDs differ structurally. Amorphous CQDs are sp^3 hybridised, while crystalline GQDs are sp^2. CQDs glow due to surface imperfections. Quantum confinement fluoresces 2–20 nm GQDs [104]. GQDs differ from CDs in that their graphene sheets are less than 100 nm and 10 layers thick. Quasi-spherical carbon nanoparticles are CDs [105]. GQDs are perfect spherical sp^2 carbon nanosheets, whereas CQDs feature a carbogenic core that mimics graphene nanolayers and amorphous carbon. Carbogenic spherical structures can have a crystalline core with both sp^2 and sp^3 carbons or a disordered structural core with mostly sp^3. Conjugated QD domains, which mimic large aromatic systems, cause bandgap transitions. In the following two portions, high-density electrons in sp^2 hybridised orbitals absorb light and generate electronic transitions. Inorganic semiconductor QDs' excellent optical and electrical properties are employed to make sensors. Carbon-based QDs offer good selectivity and sensitivity for detecting a wide range of analytes. Carbon-based QDs are non-toxic and soluble in aqueous solvents, in contrast to other QDs. Due to their exceptional optical properties and surface flaws, CQD and GQD-based nanocomposites (NCs) are perfect for sensors that detect and destroy dangerous pollutants. Since CQDs and GQDs have a limit of detection (LOD) of nanomolar, picomolar, or even femtomolar, they may be used in precision sensor systems [106]. There are sensors that are photochemical, chemiluminescent, and electrochemical. Atomic absorption spectroscopy (AAS) and inductively coupled plasma mass spectrometry (ICP-MS) are two expensive and rigid detection methods for metal ions and chemical species. Photoluminescence-based sensors are better for these kinds of applications because of their low cost, simplicity, high sensitivity, and selectivity. Low LODs are one benefit of CQD and GQD fluorescence sensors [107]. If the LOD is low, the sensor is extremely sensitive. CQDs and GQDs photoluminescence sensors have strong selectivity, which enables them to identify certain ions or chemical compounds in a mixture of other substances. CQDs-based sensors have identified Fe^{3+} [90], Hg^{2+} [108], Pb^{2+} [109], and organophosphorus pesticides [27].

Because of its low equipment requirements, high sensitivity, broad linear range, and lack of background scattering light, chemiluminescence sensors are becoming more and more common in sensing applications [110]. Fe^{2+}, Cu^{2+}, H_2O_2, and 4-nitrophenol have all been detected using chemiluminescence sensors [111–114].

Because of its easier setup, higher sensitivity, and label-free nature, electrochemiluminescence (ECL), often referred to as electrogenerated chemiluminescence, has

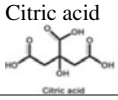
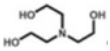
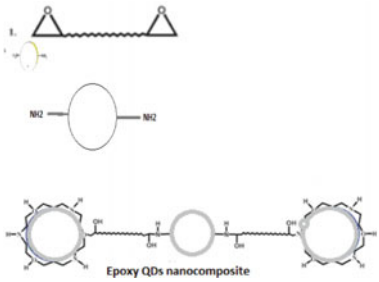
found increased application in analytical chemistry. In order to illustrate the ECL emission feature required to create ECL sensors, researchers demonstrated a variety of semi-conductor nano-crystals, such as QDs, CQDs, and GQDs. Reaction location and timing may be regulated when light-emitting samples are produced in situ, close to the electrode surface, because the ECL has nearly no background [19]. Ag/CQDs have property mutability. Hg^{2+} ions transform into Hg and break down into small fragments that combine with Ag NPs to create an AgHg amalgam layer on the surface, which reduces absorbance. Ag/CQDs' CQDs expedite the electron transfer from Ag NPs to Hg^{2+} ions. Ag/CQDs' CQDs expedite the electron transfer from Ag NPs to Hg^{2+} ions. L-tryptophan capped CQDs (L-CQDs) in aqueous solution were employed by Wan et al. [1] to detect Hg^{2+} ions (LOD of 11 nm) in 1 min. An electronic structural alteration was induced in L-CQDs via interactions between the amino, carbonyl, and Hg^{2+} moiety. Excitons recombine as a result of the charge transfer process, which lowers fluorescence emission. To identify Pb^{2+} ions in porphyria, copper nanoclusters@nitrogen-doped carbon dots (CuNCs-CNQDs) were employed. The self-calibration signal was provided by CNQDs by preserving fluorescence in Pb^{2+}. CuNCs-CNQDs nanohybrids, with a fluorescence intensity ratio difference between fluorophores-based LOD of 0.0031 mgL-1, were employed as ratiometric probes to detect Pb^{2+} at concentrations ranging from 0.010 to 2.5 mgL-1. The sensor detected Hg^{2+} in seafood with 86.45–114.47% recoveries and 0.20–1.92% RSDs. It was also accurate, dependable, and selective. The detection of heavy metals [115], glucose [116], pesticides [117–122], DNA [23], and nitro-explosives [123].

6 Epoxy-Graphene Quantum Dots Nanocomposites

In order to create graphene quantum dots, multiple weight percentages of graphene were introduced to epoxy resin along with a curing agent. This demonstrated the reinforcing effects of graphene quantum dots on different characteristics of epoxy resins. The resulting nanocomposites were characterised using FTIR, photoluminescence, UV-visible absorption, FESEM, TGA, tensile testing, and DMTA analysis. These techniques demonstrated that the incorporation of graphene quantum dots into the epoxy resin improved the nanocomposites' mechanical characteristics, viscoelastic properties, and thermal stability. Table 2 illustrates the epoxy graphene quantum dot fabrication process.

The excitation and emission peaks of GQDs and Ep/GQDs nanocomposites were detected at 350 nm and 450 nm, respectively, according to their FTIR Photoluminescence (PL) spectra. The photoluminescence curve indicates that when the electrons in the functional groups C=O and C=C on the edges of GQDs are excited, they may produce strong visible radiation [124]. According to research on the Ep/GQDs nanocomposites' fluorescence characteristics, excitation happened about 355 nm, and the emission peak happened at about 460 nm. The data indicate a strong correlation between the quantity of GQDs incorporated into the polymer matrix and variations in emission intensity. This is because there is a direct correlation between

Table 2 Synthesis of epoxy graphene quantum dots through series of different steps of potential chemical reactions [125]

Steps of synthesis of epoxygraphene QDs	Analyte	Process	Resultant product
(i)	Citric acid 	Microwave irradiation 800 W, 7 min	Graphene quantum dots (GQDs)
(ii)	GQDs	Rotary operation for 1 h, Oven 12 h, 60^0C at 7 pH 	Epoxy graphene quantum dot
(iii)		Chemical interaction with polymer matrix 	

the volume of GQDs in the polymer matrix and their increased fluorescence. The mechanical property data indicates that when the filler content increases, the tensile strength of the nanocomposites increases due to the addition of GQDs nanoparticles in the polymer matrix. Comparing Ep/GQDs 5 wt% to the other samples, the results show that it has a higher tensile strength. Numerous reasons might account for the reported rise in nanocomposites' tensile strength. These include the strong chemical interactions that take place between the GQDs and epoxy matrix, as well as the uniform dispersion of nanoparticles in the polymer matrix. Van der Waals forces, hydrogen bonds, and covalent bonds are a few examples of these interactions that work to fortify the nanocomposites and enhance their mechanical qualities. In particular, covalent interactions arise between the curing agent and epoxide groups, while hydrogen bonds form between the epoxy and carbonyl, carboxyl, and hydroxyl groups on the margins of the GQDs. DMTA analysis was used to analyse the viscoelastic characteristics. The results showed that the presence of GQD nanoparticles in the epoxy structure led to strong chemical interactions between the polymer matrix and the nanoparticles. The nanoparticles also filled up gaps and previously empty regions. It was uncovered that the storage modulus has improved.

7 Zinc Oxide Quantum Dots/Graphene Nanocomposites

Using a straightforward solution-based method, graphene nanocomposites embellished with unique zinc oxide quantum dots (ZnO QDs) were created. Nucleation and development on the surface of a graphene template create zinc oxide quantum dots (QDs), with a size of around 5 nm. By varying the reaction duration and precursor concentration, the density of their dispersion may be easily regulated. At room temperature, the formaldehyde sensing properties of pure graphene are enhanced by a factor of 4 when incorporated with ZnO QDs to form a nanocomposite material. In addition, the nanocomposite-based sensors exhibit rapid response (approximately 30 s) and recovery (approximately 40 s) characteristics, as well as exceptional selectivity and stability at ambient temperature. The synergistic interaction between ZnO quantum dots and graphene is responsible for the reported increase in gas sensing. The oxidation process of the gas under study on the surface of ZnO QDs is responsible for the electron transfer phenomena between ZnO quantum dots (QDs) and graphene. DRIFT spectra results demonstrates the reliability of the proposed gas sensing mechanism. The utilisation of ZnO quantum dots (QDs) in conjunction with graphene nanocomposites as sensors presents promising prospects for the surveillance of atmospheric contamination, particularly with respect to volatile organic compounds (VOCs) that are hazardous and toxic. As previously documented, graphene has demonstrated the capability to detect various types of gases, including but not limited to NO2 and CO. The present study reports on the gas sensing properties of a nanocomposite consisting of ZnO quantum dots and graphene at room temperature. The targeted gas molecule for detection was formaldehyde (HCHO).

8 Chitosan Based Carboxymethyl Nanocomposites with Chemically Bonded Quantum Dots

Using carboxymethyl chitosan (CMCS), a biocompatible nanocomposite consisting of magnetic nanoparticles (MNPs) and fluorescent quantum dots (QDs), magnetic-fluorescent nanoparticles (MFNPs) have been produced. Covalent bonds are an efficient way to connect magnetic nanoparticles (MNPs) and fluorescent quantum dots (QDs) to the carboxymethyl chitosan (CMCS) surface. The composite exhibits high colloidal stability in physiological conditions and keeps its advantageous magnetic and fluorescence features. The CMCS composite was used to build nanocarriers that had minimal cytotoxicity, prolonged drug release, and pH-dependent drug release characteristics. The reported CMCS composites hold great potential for effective cancer targeting, medication delivery, and in situ cellular imaging. These MFNPs effectively conjugate QDs and MNPs to the surface of CMCS through covalent bonding. The composite exhibits high colloidal stability in physiological conditions and keeps its advantageous magnetic and fluorescence features. Folate (FA) was added to the nanocomposites to provide a delivery system with a targeting function.

FA is a particular targeting ligand. Doxorubicin (DOX), a chemotherapy medication, was loaded to provide the therapeutic effect through electrostatic and hydrophobic interactions. The overall DOX release profile indicates pH sensitivity. Both confocal laser scanning microscopy examination and flow cytometry analysis indicated that these nanocomposites were taken up by cancer cells via FA receptor-mediated endocytosis pathway. Thus CMCS-based nanocomposites created in this study show enormous promise for efficient drug administration, cancer targeting, and in situ cellular imaging.

Nanocomposites with several functional features are created by combining nanomaterials with various physicochemical properties. For drug delivery and cell monitoring, a lot of work has recently been put into designing and creating nanocomposites using fluorescent QDs and magnetic nanoparticles (MNPs) [126, 127]. Opportunities for controlled drug administration and release, multimodal bioimaging, selective cell targeting, and diagnostic and therapeutic capabilities are presented by the resultant composites [128]. Such multi-functional composites are frequently created via.

Effectively, surface-modified QDs, MNPs, and folateFA have been combined with CMCS matrix to form nanocomposites for targeted drug delivery and cellular imaging. The presence of -COOH and -NH2 groups in CMCS allows for the formation of amido linkage (-CONH-) with QDs, MNPs, or foalte. In contrast to physical interactions like electrostatic force, strong chemical bonds between the constituents ensure the stability of the generated nanocomposites under physiological settings. The resulting composite possesses both the advantageous magnetic properties of MNPs and the strong fluorescence of QDs. Some possible advantages of in vitro research include low cytotoxicity, delayed and pH-dependent drug release patterns, high cellular uptake efficiency, and receptor-specific endocytosis.

9 CdS and CdSe Quantum Dots in Poly (3-Hexylthiophene) Based Nanocomposites

The efficiency of optical stimulation to form electron hole pairs and the accompanying radiative decay may be controlled by varying the size, shape, and distribution of nanoparticles because of the quantum size effect [129]. The effectiveness of optical excitation is controlled by quantum dot-polymer interactions as a result of the impacts of quantum size. Nanocrystal and polymer mixtures have shown promising breakthroughs in solar technology. When the nanocrystal has a notable bandgap, both the conjugated polymer and the nanocrystal contribute to the absorption in composites. Excellent hole-transporting polymers, such poly(3-hexylthiophene) (P3HT), can be combined with electron-transporting nanocrystals to take advantage of inorganic semiconductors' high transport capabilities without sacrificing organic materials' ability to be processed in a solution. An appreciable portion of solar energy is absorbed by composite devices that incorporate inorganic nanocrystals because they have a greater affinity for electrons than conjugated polymers. Conducting

poly(3-hexylthiophene) (P3HT) matrix has been used to create CdS and CdSe nanoparticles. The spectroscopic results demonstrate that the electrical structure of the polymer is significantly influenced by the characteristics of the inserted semiconducting nanoparticles. SEM and TEM images show the ordered shape of the CdS and CdSe nanoparticles in the presence of the polymer matrix. P3HT shows a prominent absorption band at 451 nm in the superimposed UV-vis spectra of (A) P3HT, (B) P3HT/CdS, and (C) P3HT/CdSe nanocomposites, which may be caused by the excitation of electrons in the p-conjugated system. Similarly, CdS and CdSe nanocomposites show higher intensity absorption bands at 448 and 446 nm, which correspond to band gaps of 2.76 and 2.78 eV, respectively, suggesting that the p-p* transitions of conjugated systems are more prominent.

There is a modest blue shift of 3 and 5 nm, respectively, following the introduction of CdS and CdSe nanoparticles, indicating that the band gap in the composites is larger than that of pure P3HT. Short-range optical absorption curves offer theories on the development and synthesis of nanoparticles. The structure and apparent morphology of the nanocomposites differ significantly from those of the polymer, as shown by SEM pictures of the polymer matrix. Similar nanoparticles with consistent interparticle separation are seen in the CdS/P3HT nanocomposite, but pores with distinct edges and varying distribution are seen in the polymer. Interestingly, the CdSe/P3HTsample exhibits somewhat larger particles with variable inter-particle spacing. These characteristics clearly demonstrate the trapping of nanoparticles in the polymer matrix and the apparent shape supports nanocrystal-polymer composites of greater sizes.

TEM scans reveal a few more peaks, which are related to the P3HT polymer's increased crystallinity as a result of the nanoclusters' existence. To sum up, P3HT serves as an effective matrix for encapsulating CdS and CdSe nanoparticles in terms of physical, thermal, and electrical properties. This is particularly useful for constructing various devices intended for optical and optoelectronic uses. Morphological investigations using SEM and TEM show the development of nanocomposites with ordered Q-dots with an average particle size in the range of 3–5 nm. UV tests showed that the conjugation present in the polymer matrix creates considerable overlapping between electron-hole pair wave functions, despite TGA measurements demonstrating the nanocomposites' greater heat stability. A comparison of the cyclic voltammograms for pure polymer, CdS/P3HT, and CdSe/P3HT nanocomposites in the presence and absence of light demonstrates the capacity of the quantum dots immobilised in polymer to trap the intermediate radical cation at a given potential.

10 Poly(Methyl Methacrylate)/ZnO Nanocomposites Based on KH570 Surface Modified ZnO Quantum Dots

Transparent luminous ZnO embedded PMMA polymer has been generated by grafting poly(methyl methacrylate) (PMMA) onto the surface of ZnO QDs after treatment with g-methacryloxypropyl trimethoxy silane (KH570) [130]. The finished ZnO/PMMA nanocomposites with KH570-modified nanoparticles show better dispersibility while retaining the ZnO nanoparticles' enhanced luminosity. The PMMA/ZnO nanocomposite films that are generated have great UV shielding efficacy, enhanced thermal stability, and good transparency.

The characteristic absorption peaks of $–CH_2–$ stretching vibration, CO stretching vibration, –SiOH stretching vibration, Zn–O–Si, and Si–O–Si symmetrical stretching vibration at 2953.92, 1700.43, 1184.56, 943.47, and 817.71 cm^{-1} are obtained at 3394.35 cm^{-1} due to hydroxyl absorption. These findings demonstrate that the introduction of active groups onto the surface of the nanoparticles led to the hydrolysis-condensation formation of a thin layer of organic silica nanonetwork. The FT-IR spectra of ZnO nanoparticles modified by KH570 following PMMA grafting are displayed in Fig. 1c. The C O stretching vibration and CH3 stretching vibration from PMMA are shown by the lines at 1735 and 1454 cm^{-1}. Stretching vibration bands of PMMA C-O-C are located at 1148, 1197, 1246, and 1283 cm^{-1}. It demonstrates how PMMA was grafted onto ZnO nanoparticles by hydrolysis–condensation, forming a thin layer of organic silica nanonetwork.

Transmission electron microscopy (TEM) provides evidence for the concept that KH570-surfaced modification and polymer play a major role in stabilising and spreading the nanoparticles because of the covalent link that occurs between them.

11 Nanocomposites Based on an Oxadiazole–Carbazole Copolymer (POC) and InP/ZnS Quantum Dots

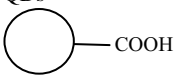
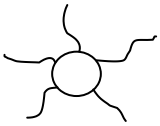
Derivatives of carbazoles are used to modify the emission characteristics of polymers in the CIE diagram, therefore prolonging the life of devices. These derivatives produce a strong blue emission because they have an electron transporter unit (oxadiazole) and a hole transporter unit (carbazole). Studies [131], Peng and Zhang (1999), Bugatti et al. (2006), Acierno et al. (2002, 2003, 2004), Concilio et al. (2010) have all reported on this. The POC polymer has intercalated aliphatic lengths of active oxadiazole/cabazole units, which improve solubility in a range of organic solvents and guarantee favourable film shape. The two different kinds of red emitters QDs, InP/ZnS(570) and InP/ZnS(627), which are referred to as POC-InP/ZnS(570) and InP/ZnS(627), were combined to create the nanocomposites. The pure polymer, pure InP/ZnS(570) quantum dots (QDs), and nanocomposites with QDs at 25, 50, and 75 weight percent (wt%) were all subjected to Fourier-transform infrared (FTIR) spectra. The carbazole/oxadiazole rings' aromatic C-H signals may be detected at

2928, 2853, and 1758, 1609, and 1488 cm^{-1}, in that order. Oleylamine-capped InP/ZnS quantum dots (QDs) have a few unique peaks in their infrared spectra, and these peaks are positioned similarly to those of the POC QDs that have been investigated earlier. These peaks are specifically seen at 1,461 and 1,563 cm^{-1}, which represent the oleylamine's N–H bending, and at 2,846 and 2,922 cm^{-1}, which represent the C–H stretching. The assessment of POC's valence (HOMO) and conduction (LUMO) energy levels verified the viability of an efficient electron/energy transfer between POC and QDs. There seems to be a substantial preference for the flow of electrons and energy between the two materials. The POC-InP/ZnS(570) Nanocomposite's spectra show that peaks linked to the emission of POC and QD coexist. Concurrently, we find that the polymer peak in every nanocomposit has a little blue shift when compared to the pure polymer. Furthermore, when the concentration of QD rises, the polymer peak's intensity decreases. The concentration of QDs in the nanocomposites positively correlates with the intensity of the QD emission peak, which is detected at a wavelength of 655 nm. The facts stated above suggest that the bulk material's polymer chain organisation is altered by quantum dots (QDs). Moreover, the polymer and the nanoparticles have an efficient energy transfer mechanism. The study displays the POC-InP/ZnS(627) nanocomposites' photoluminescence spectra, which have been normalised according to the quantity of photons absorbed at the excitation wavelength. At 400 nm, the nanocomposites were energised. A main peak that is stimulated at 400 nm is visible in the emission spectrum. Emissions have also been detected at four different wavelengths. Changes in polymer organisation brought about by an increase in QD number might be the cause of the observed increase in polymer fluorescence lifetime. This ultimately causes the polymer's relaxation decay pathway to emerge in a unique way. Quantum dots (QDs) are much smaller than the extended polymer chains, which makes it easier for them to be incorporated into the chains. This event also suggests that a high efficiency of energy transmission to the QDs can be achieved.

12 CuInS2 Quantum Dots@Graphene Oxide Nanocomposites

The fabrication of the Ky2-CuInS2 quantum dots (QDs)@graphene oxide (GO) nanocomposites involved the conjugation of amino terminal kanamycin-binding Ky2 aptamer with carboxy groups present on the surface of CuInS2 QDs, which were modified using mercaptopropionic acid. N-hydroxysuccinimide and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride were present when this was accomplished. The nucleobases and graphene oxide (GO), the Ky2-CuInS2 quantum dots were easily attached to the GO surface by pi-pi stacking contact. The "turn-off" and "turn-on" fluorescence characteristics of Ky2-CuInS2 QDs are used by the sensor. For kanamycin, under ideal circumstances, a linear response was found in the range of 0.3–45 nmol L^{-1} (0.174–26.1 mg L^{-1}), with a low detection limit of

Table 3 Tabular illustration for fabrication of Ky2-CuInS2 QDs (Liu et al. 2016)

Various steps for fabrication of Ky2-CuInS2 QDs	Process	Product
Step 1—CulnS2 QDs 	Conjugation of carboxy groups present on the surface of CuInS2 with mercaptopropionic acid, with amino terminal kanamycin-binding Ky2 aptamer in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and N-hydroxysuccinimide	 Ky2 CulnS2 QDs
Ky2 CulnS2 QDs	Affixed on graphene oxide (GO) surface with/without kanamycin	Ky2 CuInS2 QUANTUM DOTS@graphene oxide nanocomposites

0.12 nmol L^{-1} (0.070 mg L^{-1}), exceeding the MRL set by the EU. Table 3 shows the tabular representation used in the Ky2-CuInS2 QD production process.

13 Polyurethane-Based Bulk Nanocomposites from 1-Thioglycerol-Stabilized CdTe Quantum Dots

N_2H_4-enhanced growth promotes the production of CdTe quantum dots (QDs) in an aqueous solution. Thioglycerol (TG) stabilised the QDs, and their photoluminescence enhanced. The synthesis procedure led to a significant decrease in the time scale of the size evolution of the quantum dots. The process made it easier for QDs to develop at room temperature and lower temperatures, which prevented TG from decomposing and sulphur from becoming trapped inside the QDs. The QDs' hydroxyl coverage from the TG ligand made them miscible with PU prepolymers in dimethyl sulfoxide. Bulk nanocomposites were formed when hydroxyl coverage enabled the bonding of polyurethane (PU) to the surface of quantum dots (QDs) by the interaction of hydroxyl (-OH) and isocyanate (-NCO) groups resulting in the formation of bulk nanocomposites of CdTe QD-PU. These luminous nanocomposites are made of prepolymers and CdTe QDs stabilised with thioglycerol (TG). An important part was the optimisation of the TG-stabilized QD synthesis process, which was accomplished in an aqueous solution via a N2H4-promoted method. This method produced a notable improvement in the PL intensity as well as a significant decrease in the preparation time. Because of the hydroxyl coverage from TG, QDs and PU prepolymers in DMSO were miscible without the need for ligand exchange. This made it possible for the reaction between -OH and -NCO to initiate polymerization. More investigation revealed that the polyurethane (PU)-based nanocomposites included an equal distribution of quantum dots (QDs). The QDs' size-dependent

photoluminescence (PL) characteristics were also preserved. The ease and feasibility of synthesising TG-stabilized QDs and polymerizing them currently suggests that luminescent nanocomposites could have practical applications in the display and optical fields.

14 Conclusion

The features and uses of the newly developed nanocomposites based on quantum dots as sensors have been covered in length in this review chapter. However, research into nanocomposites based on QDs is still in its early phases. Even though there has been a lot of study on synthesis approaches over the past ten years, it is still challenging to discover a scalable, effective, yet straightforward manufacturing process for their high-quality synthesis. And even other features require attention.

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Quantum Dots/Polymer Composites

Soumita Talukdar and Amit Gupta

Abstract Quantum dots (QD) are semiconductor nanoparticles which exhibit exciting quantum mechanical properties at a macro scale, such that they are often touted as the "macroscopic atoms". However, owing to their sizes, real world applications often need to cast them in a bulk object or anchor them to a substrate. Most common approaches include casting as a film or making composites. In this chapter we will discuss one such approach of casting the QD in a polymer matrix. This approach is flexible, easy to synthesize, and provides exceedingly useful applications in the field of analytical chemistry, photoluminescent materials, catalysis, and biologically useful materials.

Keywords Quantum dots · Polymer · Nanocomposite · Optoelectronics · Sensor

1 Introduction

Recent emergence of quantum dots (QD) as the magic material is being established for their unique physical properties, tunable surface properties, advanced synthetic routes and vast array of applications [1]. Moreover, colloidal-quantum dots are emerging to be a new branch of research domain as 2357 review work have been reported according to Scifinder®, since 2012 [2]. The applications ranges from optoelectronics, photocatalysis, fluorescent sensors, drug delivery, gamma scintillation, energy storage, solar cells, etc. [3–7]. Higher charge-carrier mobility, generation of multiple exciton ability, tunable surface and band structure properties make QDs an ideal candidate for photovoltaic applications [8]. Photoluminescence (PL) is the most dominant characteristics that is extensively studied [9]. The absorbance and emission characteristics of various polymers and their composites are shown in Table 1. In

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addition, high surface-area to volume ratio and tunable redox potentials enable these QDs highly effective as catalysts for photo-induced redox reactions [10]. Varied sizes of QDs can be prepared by modification of parameters such as temperature, reaction time and synthetic routes, enabling desired applications [1]. Thereby enabling array of QDs excited at single wavelength with multiple well-separated emissions. However, this phenomenon may also lead to energy loss and emission re-absorption [11], phenomenon known as the inner filter effect. An interesting property of inner filter effect (IFE) was also observed in core–shell quantum dot structures, where the photo luminescence (PL) of smaller QD was self-absorbed by larger QDs exhibiting a red shift of photoluminescence. Therefore, these QD based nanocomposites can be considered as potential candidate for exploitation of IFE and develop spectroscopic displacement sensors [12].

Composites of such QDs, significantly improve the inadequacies of QDs, while restoring their original diverse properties. Due to the presence of diverse functional groups on the surface of QDs, they readily form composites with improved dispersibility [9]. Thus, properties such as optical absorptivity, photobleaching resistance, low toxicity, chemical stability, bio-compatibility and hydrophilicity can be highly alleviated in the QD/polymer composites. Development of polymer based QD materials proved to be a boon in various fields. Third generation solar cells, mainly comprised of inorganic–organic, polymer based nanocomposites [13]. Nanoparticles

Table 1 Absorbance and emission characteristics of various QD/polymer composites [9]

QD/polymer composite	Absorbance (nm)	Emission (nm)	Emission color	QY (%)	Ref.
PVA-N@C-dots	286, 355	~460	Cyan	44	[17]
N-doped CD in chitosan/PVA	360	436	Cyan	–	[18]
CD in polyethylene glycol	200–500	550	Yellow	14.86	[19]
Magnetite-MIPs	–	470	Blue	–	[20]
Cu-alginate	–	513	Green		[21]
C-dots/PVB film	353, 410, 500	550	Green–Blue, Orange–Red		[22]
CD polymer	200–500	550	Yellow	14.86	[23]
CDs@MIPs	250–300	450	Blue		[24]
WCDs@polystyrene		~590	Orange, Blue	10.7, 15.2	[25]
CD in polyethyleneimine	290, 340, 380	~565	Cyan	1.9–4	[26]
C-dots/PVA 360	282, 341	459	Green	8.64	[27]
CDs@PVA	~350	420–440	Blue		[28]
Poly(VPBA-AAm)	241	515	Blue		[29]
CQD/PAN nanofibers	314, 316, 318	530 560, 598, 660	Red, Green, Blue		[30]

(NP) have enabled modification and improvement in properties of polymer matrix [14]. NP reinforced polymer matrix exhibited enhanced mechanical, thermal and electrical properties which largely depends on the polymer-NP interface, concentration and uniformity of NP added. For specific and selective sensing probes, molecularly imprinted polymer (MIP) have gained much attention recently, due to the generation of specific-recognition sites or cavities during synthesis [15]. Conducting polymers on the other hand are extensively used as electrode materials due to their interactions capability such as H-bonding and π-π interactions, ion-exchange ability, hydrophobicity and presence of multi-functional groups [16].

With ever emerging applications and possibilities, QD/polymer composites have unlocked path for next generation materials. We are already witnessing breakthroughs of such materials in consumer electronics, sensors and bio-imaging applications. This chapter will discuss in detail about the nature, synthesis and applications of such composites.

2 Synthesis

Developing a highly efficient QD/polymer composite is very crucial. Fabrication of polymers with particles such as QDs, enables greater strength and stability. The QDs can also be stabilised by polymer encapsulation. Common techniques used for the synthesis of polymer nanocomposites are solution mixing, melt mixing, in situ particle processing, in-situ polymerization, template synthesis, grafting, electrohydrodynamic, etc., depending upon the types of QDs and polymers and their interactions [31]. There are mainly two synthetic approaches for synthesizing QDs, namely the (a) top-down and (b) bottom-up approach. The physio-chemical properties significantly depend on the type of precursor and the synthetic technique. The top-down approach is beneficial in case of large-scale production and oxygen-functionalised surface, whereas bottom-up approach facilitates the size, morphology and surface functionalised tailored synthesis [9]. The various synthetic methods used for preparing QD/polymers composites are discussed.

2.1 Solution Mixing

A magnetic and photoluminescent nanocarrier Fe_3O_4-HBPLCArg/QDs was developed by mixing dispersed CD solution in DMSO and N,N-dicyclohexylcarbodiimide (DCC), 4-dimethylaminopyridine (DMAP) with Fe_3O_4-HBPLC (hyperbranched poly L-lysine citramid) for 24 h [32]. QD dispersed piezoelectric polymer films were synthesized by mixing ZnO-D capped and stabilized by dopamine, in a mixture of acetone and DMF, with polyvinylidene fluoride-co-hexafluoropropylen (PVDF-HFP) copolymer, stirred for 12 h, followed by spin-coating over flexible electrode

for 60 s at 1000 rpm. The hybrid film was then annealed for 1 h, at low temperature below 130 °C to avoid melt or delamination [33].

2.2 Template Synthesis

For synthesis of functionalised QD/polymer composites hard and soft template technique can be employed. Soft templates are easy to dissolve and economical whereas the hard templates used provides shape and size controlling ability and uniform dispersion [10]. In soft templates such as self-assembled biopolymers, water emulsions, soft carbon, surfactants or oil are commonly used. SnS_2 QDs/pg-C_3N_4/MWCNT (multi walled carbon nanotubes) surface was imprinted with 3-aminopropyltriethoxysilane (APTES) polymer resulting in Molecularly imprinted polymer on SnS_2 (MIP@ SnS_2) QDs/pg-C_3N_4/MWCNTs nanocomposite by sol–gel method. Indoxacarb (IDC) is used as soft template which can be easily dissolved by a solvent mixture of ethanol/acetonitrile (8:2 v/v)0 [34]. On the contrary, hard rigid structures are used as hard templates. Facilitates the shape and size tuning of nanocomposite synthesis. Hierarchical structure of $CsPbBr_3$ QDs embedded in di-block, amphiphilic copolymer polystyrene-poly2-vinyl pyridine (PS-b-P2VP, S2VP), was achieved using S2VP as micelle template, in which the pyridine group closely binds to $CsPbBr_3$ facilitating in-situ growth. The nanocomposite was synthesized by spin-coating of the mixture of $CsPbBr_3$ QD and S2PV polymer in toluene, followed by annealing under mild temperature [35]. HA-GQD-MIP nanocomposite probe was developed using Norfloxacin as template, which thereby create norfloxacin specific cavities in the synthesized MIP, shown in Fig. 1 [15].

2.3 Grafting

Various functionalised polymer coated CdSe QDs core–shell nanocomposite synthesis was reported by Zhu et al., a light-mediated polymerization, facilitating one-pot synthesis of QD/polymer nanocomposites [36]. CdSe QDs acting both as a catalyst as well as the inorganic core to the organic–inorganic core shell structure. This approach can be highly beneficial to synthesise and tune the polymer functionality and can be used for developing complex hybrid nanomaterials. Recently thiol-ene photo-polymerization have been reported to be highly efficient and instantaneous crosslinking method unaffected by ambient reaction environment. Here nanoparticle ligand act as the site from where the step-growth polymerization initiates resulting in crosslinked composites. Other photopolymerization was also reported initiated by UV light over QD site for efficient LED applications. Fabrication of Blue LED was also reported by blending red and green QD in appropriate ratio, with monomer, oligomer along with the photoinitiater [37]. By incorporation of QDs in polymer matrix, significant improvement of thermal stability was observed.

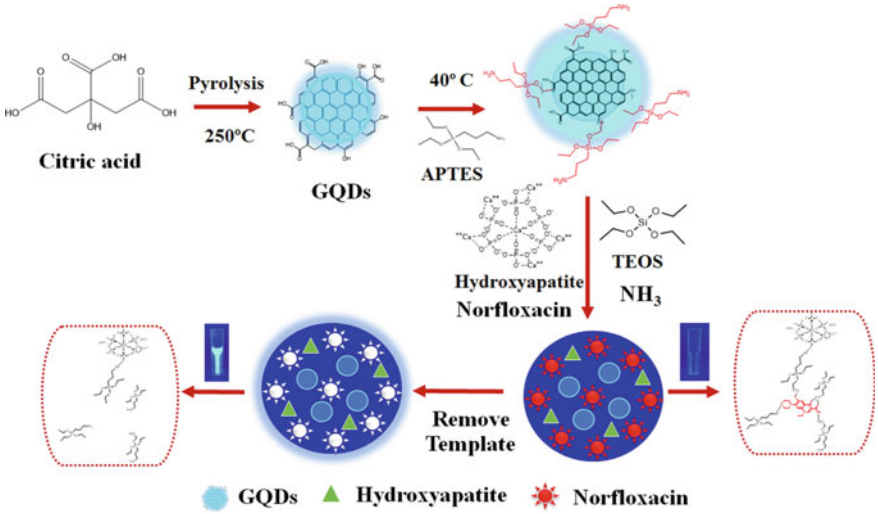

Fig. 1 Fabrication of HA-GQD-MIP nanocomposite using Norfloxacin template for Norfloxacin specific detection. Reproduced with permission from *Microchemical Journal, 2020, 158, 105,127* [15]

Thiol-ene crosslinking was also reported to be initiated thermally forming $CsPbBr_3$ QD-Polymer self-healing, elastic and luminescent composite [38]. Figure 2 also lists schematic for grafting QD with bidentate ligands [1].

2.4 Hydrothermal/Solvothermal

A photoluminescent nanocomposite was prepared using arginine derived hyperbranched polymer and glucose derived QD by simple hydrothermal method in a stainless steel autoclave at 180 °C for 6 h [39]. This nanocomposite was used as nano carrier in drug delivery. Green QDs such as ZnCdSeS/ZnS and Red QDs such ZnCdSe/ZnS encapsulated in polypropylene (PP) thermoplastic matrix, composite film exhibited color conversion property with high stability as compared to conventional PMMA film. Besides providing better protection from oxygen and moisture, PP polymer matrix also rendered a mild environment due to the absence of ester groups, as compared to PMMA matrix [40].

2.5 Electrochemical Method

A liquid phase synthesis of nanocomposite via electrohydrodynamic emulsification mediated by self-assembly approach was reported by Lee et al. [41] Here a

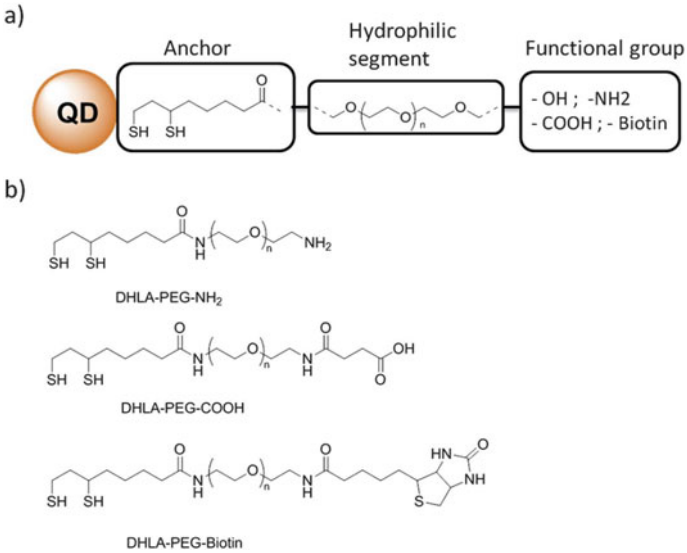

Fig. 2 **a** Schematic of grafting to the surface of QDs using bidentate ligands. **b** The end-functionalized polymers with suitable chemical groups for further derivatization, reproduced with permission from *Nanoscale, 2013, 5, 12,018*

non-conductive fluid chloroform containing poly(styrene-b-ethylene oxide) functionalised with amphiphilic block copolymer octadecylamine is being sprayed into a conductive fluid distilled water containing CdSe/ZnS QD, in between a metal needle and a counter electrode. The size of the emulsion droplets decreased with the increased voltage. On the other hand, polymerization of thionine was facilitated by amino-functionalised GQD (afGQD), to produce poly(thionine) modified afGQD nanocomposite electrochemically, due to their improved electro-conductivity [42].

2.6 Chemical Oxidation

Chemical oxidation is another simple in-situ synthesis of QD@polymer nanocomposite. Polyaniline@GO nanocomposite was synthesised by polymerization of aniline in presence of oxidising agent ammonium persulfate (APS) and acidic medium [16, 43].

2.7 Wrapping

Strategy to functionalise nanostructures using non-covalent interaction between organic polymers and inorganic QDs. A nano-bio catalyst graphene oxide/glucose oxidase was synthesised by wrapping enzyme molecules over graphene oxide surface via adsorption and crosslinking [44].

2.8 Sol–gel Method

One of the simplest, low cost and greener method of polymerization under mild conditions. Non-imprinted polymer NIP@SiCQDs and molecularly imprinted MIP@SiCQDs nanocomposite was reported to be synthesized using this method, coupled with template synthesis technique, using target analyte as template for synthesizing MIP@SiCQDs. SiCQDs were first functionalized by 3-aminopropyltriethoxysilane (APTES), then MIP and NIP nanocomposite was prepared by sol–gel method, using APTES monomer and tetraethoxysilane (TEOS) as cross-linker [45].

3 Reinforcements, Design, and Fabrication

3.1 Surface Fabrication of QDs

Formation of efficient and specific QD/polymer nanocomposites requires good dispersibility of QDs in the solvent with minimum aggregation. Good dispersibility can be commonly achieved by exchanging hydrophobic ligands with a chemical group with high affinity towards the binding QD. Amphiphilic ligands can also be coated over the QDs, the hydrophobic part easily binds to non-polar solvent, simultaneously the hydrophilic part renders the QD soluble in water. CdSe/ZnS QDs were coated with designed peptide comprising of a hydrophilic part for dispersion in water and an adhesive part, which binds to the QD [55] (see also Fig. 3).

Multidentate ligands also enhances the binding affinity with the QDs due to the presence of multiple binding sites, which thereby results in the QD/polymer interface stability [1] (also Fig. 2).

Results show that the chain length of the dispersant polymer also play a significant role in the dispersibility of the QDs due to effects of static and steric stabilization [31]. Silane modified ZnO QD-silicone nanocomposite exhibited slightly lower transmittance than that of pure silicone polymer, which suggests excellent dispersibility of QD over silicone matrix.

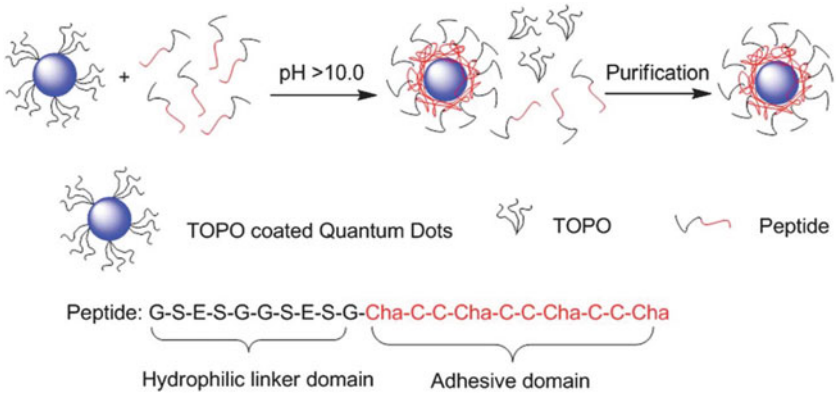

Fig. 3 CdSe/ZnS QDs with amphiphilic peptide ligand with an adhesive part to bind to QD and hydrophilic part to promote water dispersibility, reproduced with permission from, *Nanoscale, 2013, 5, 12,018–12,032*

3.2 Composite Fabrication

Polymer matrixes have wide range of applications and have replaced conventional materials such as ceramics, metals, alloys mostly due to their low cost of production and lightweight property. However, these polymer matrices have certain drawbacks such as thermal, chemical and photo instability [56]. Particle reinforced polymer matrix especially with nanoparticles or QDs are reported to be significantly beneficial in improving the stability of such polymer materials. Recently multi-scale composites attracted significant attention due to their advanced multi-functionality and improved electrical, mechanical, and optical properties. They are superior composites which are reinforced with nanoscale particles and macroscale fibres. The most common nanoparticle reinforced materials that are widely used are mainly carbon nanotubes (CNT), graphene, metal oxides, metals, etc. The key to effective reinforcements is uniform dispersion of the QDs into the polymer matrix and their stabilization from agglomeration. Ultra-sonication is most widely used mechanical technique for QD dispersion. Both ultra-sonication and ball-milling use shear forces for the dispersion of QDs which can also results in deformation of the original particles, generation of heat, defective structure, etc. Cryo-milling improved the thermal and electrical properties under lower temperatures.

To overcome the challenges of the mechanical techniques, low-cost chemical techniques can be used for improved dispersion by functionalization or tuning the surface of the QDs. Large bulky groups can be attached to these QD surface to create steric repulsion, thereby inhibiting aggregation. Functionalization of QDs plays a significant role in reinforcement of the QD/polymer composites. 2D structured graphene QDs with both alcoholic and acid groups resulted in tougher and stronger graphene QD-epoxy polymer composites [14]. Oxygen, aromatic rings and amine groups facilitate the bonding of the graphene QDs to the epoxy group which can be

further associated with weak interactions such as van der Waal's interaction, hydrogen bonding, etc. Various nanoparticles which enabled reinforcement of polymer matrix are shown in Table 2 [57].

Dewetting of QD embedded in Poly(lauryl methacrylate) (PLMA) polymer films were reported to enhance fivefold thin film emission. The QD@PLMA composite was prepared by rotomixing followed by sonication. Thin films were then developed by spin-coating the composite at 30 s over silicon substrate. Controlled dewetting was then done by spraying with ethanol. Surface patterning was also done by using suitable mask and irradiating the uncovered surface with mercury lamp. This irregular film morphology led to increase in scattering of light which, thereby resulted in improved out-coupling [58]. Similarly, dewetting techniques is used for developing CdSe/ZnS core–shell structure in a polymer film for LED and Photovoltaic applications [59]. Highly efficient solar cell heterojunction was developed with high light to energy conversion, incorporating fullerene (C_{70}) covalently attached to CdSe QD blended over Poly(3-hexylthiophene-2,5-dyil) P3HT polymer.

Recently direct laser writing (DLW) technology exhibited promising tool for fabrication and processing QD@polymer nanocomposites [60]. β-carboxyethyl acrylates surface modified red CdSe/CdZnS/ZnS QDs and green CdSe/ZnS QDs was mixed with dipentaerythritol penta-acrylate (SR399) monomer resin. Polymerization was then initiated by femto second laser of 532 nm. It is a multi-photon polymerization initiated by high energy laser irradiating the QD@polymer nanocomposite. Firstly, the surface modification enabled the uniform distribution of QD into polymer matrix, secondly the size of the QD@polymer lines significantly depended on power P and velocity v.

3.3 Composite Stability

The stability of the prepared QD@polymer composite is of high significance for their optimum efficiency. Gray et al., studied the leaching of CdSe QDs from polyethylene polymer matrix in presence of common acids present in food, such as acetic acid, hydrochloric acid and citric acid. Significant leaching of QDs was observed after long term exposure to acetic acid as compared to citric which supressed the QD transfer, resulting in decrease in the PL intensity [61].

Table 2 QD reinforced polymer nanocomposites and their applications

QD	Polymer	Synthetic route	Applications	Ref.
CdSe/ZnS	Polypropylene	Mixing	–	[46]
Si capped with divinyl benzene	Polystyrene	Spin coating	Tunable refractive index	[47]
CdSe/ZnS	Polydimethyl siloxane	Ligand exchange/imprinting lithography	Photonic/optoelectronic	[44]
CdSe/CdS/ZnS	Poly(maleic acid-altoctadecene) in LDH	Drop-casting	Display/optical coatings	[48]
CdSe/ZnS	Dioctyloxybenzodithiophene-based polythiophene	Ligand exchange	Optoelectronic	[49]
PbS	Naphthalene diimide based n-type polymer	Thiol-ene	Photovoltaic	[50]
ZnCdS/ZnS QD/g-C_3N_4	Carboxymethyl Chitosan	Self-assembly	Mn^{2+} detection	[51]
CdSe/CdS	Poly(styrene-ethylene-butylene-styrene) (SEBS)	Drop casting	Displacement sensors	[12]
Ag (AMPSA)/ GO QDs	PANI	In-situ oxidative polymerization	Cr (IV) sensing	[52]
Hydroxyapetite-GQD	MIP (APTES/TEOS)	Template synthesis	Norfloxacin sensing in milk and chicken meat	[15]
GQDs@Fe_3O_4	MIP (APTES/TEOS)	Template synthesis	Ceftazidime sensing in milk	[53]
Mn–ZnS	MIP N,N′-Methylenebisacrylamide (MBA) and poly (N-isopropylacrylamide) (NIPAM) monomers	Template synthesis	Glyphosate sensing	[54]
GO	PANI	In-situ chemical-oxidative polymerization	Extraction of antibiotic tetracycline (TC)	[16]
SiCQDs	MIP (APTES/TEOS)	Sol–gel	Indoxacarb (IXC) sensing	[45]
afGQDs	Poly(thionine)	Electropolymerization	Hg^{2+} sensing	[42]

4 Applications

4.1 Light Emitting Diodes and Displays

Given the extraordinary optical properties of the quantum dots, and coupled with the fact that they can be easily tuned to entire visible spectra [62–65] unsurprisingly one of the first applications of QD-polymer nanocomposite is the high-efficiency Light Emitting Diodes (LEDs) [66]. Various QD-polymer composites have been demonstrated to have excellent yields and brightness. Usual construction of such an LED involves sandwiching a QD-polymer nanolayer between a hole transport layer for contact and conductivity (Fig. 4). These layers must be chosen carefully to ensure optimal hole-electron recombination, following which emission wavelengths can be tuned using the size and chemical constituent of the quantum dots.

Another way such composites are used in LEDs is to enhance their post hoc capabilities. e. g. using QD-polymer LED masks for white light emitting LEDs [67]. There the LED Poly(dimethylsiloxane) (PDMS) lens of a blue led was replaced with ZnCdSe/ZnSe-PDMS composites. ZnCdSe/ZnSe coreshell particles were tuned to emit in red-green region of visible spectra, rendering the LED white. There are increasing strides being made in miniaturizing these LEDs enough to develop a QD-LED based displays [62].

While, QD-LED based displays have still not found their way in the commercial market, QD-composites based displays have matured enough to be launched in consumer commercial space. Samsung electronics launched their "QLED" series of displays, which uses an composite-QD layer as a contrast enhancer on top of the LCD-LED layer [68], following which several commercial vendors have released similar displays (ViewSonic [69]). The current generation of displays use the composites-QD as a passive fluorescent layer.

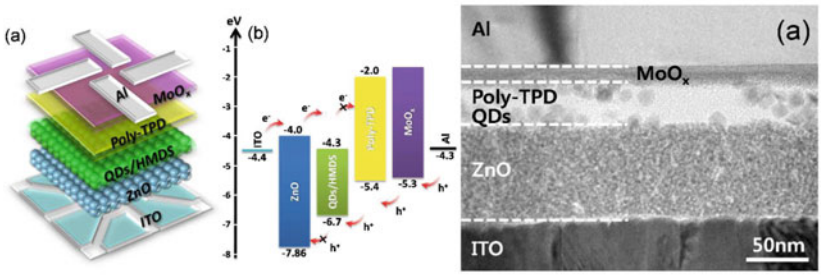

Fig. 4 QD-polymer nanocomposite LED constituting layer. Here QD-polymer composite can be seen sandwiched between ZnO and Al layers, which act as hole and electron transport respectively. Reproduced with permission from *J. Mater. Chem. C, 2017, 5, 522–526* [66]

4.2 Sensors

It was long hypothesized that due to higher optical sensitivity/variability of the quantum dots, they can be used in various sensors by the primary means of distinguishing energy flow [70]. This is primarily exploited in FRET based biological QD tags but has also resulted in large number of composite based sensors.

Simplest of them can be attributed to change-of-environment sensors, where the optical properties of QDs changes with mechanical or chemical changes in the composite film. Example of these are CdSe-polymer inkjet printed films for detecting explosives [71], measuring strength of sea-water damaged Glass Fibre Reinforced Polymer by fluorescent measurements of infused 3-(trimethoxysilyl)-1-propanethiol (MPS) modified CdS nanoparticles, and tertracycline sensor based on fluorescence quenching of B, N doped carbon dots—PVA hydrogel composite [72]. Other interesting examples includes "reverse" core–shell composite of polypyrrole/graphene quantum dots, where the polymer core is coated with graphene QDs [73]. This is somewhat different from conventional composite materials, where a polymer matrix is used to embed the active QD particle. The authors demonstrated that when these polymer graphene QD hybrids can detect Dopamine in external environments by red-shifting fluorescent emission. The change-of-environment need not come solely form direct interaction with an external chemical stimulus but can also be a result of deformation in the polymer-composite [22]. Demonstrated mechanical sensing property of carbon dots/ polyvinyl butyral composite, where the fluorescence of the c-dot/ polyvinyl butyral (PVB) film changed with the applied deformation; by measuring the relative fluorescence luminescence they were able to characterize the stress/strain of the film (Fig. 5).

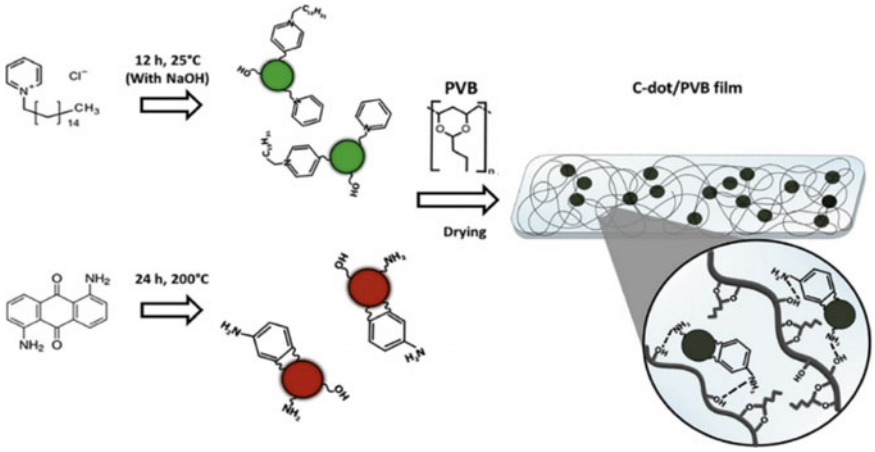

Fig. 5 Synthesis of composite film susceptible to fluorescent changes on applied strain. Reproduced from *Carbon, 2019, 152, 363–371* [22]

While most popular, optical sensing is not the only method to detect the change of chemical environment. Several reports exist, for example, detecting Metobromuron herbicide in aqueous media using gold-nitrogen doped carbon quantum dots with silver core–shell polymer composite [74]. Using polypyrrole/graphene quantum dots composition [75] reported electrochemical sensing of bisphenol A.

QD based sensors are not limited to the change-in-environment sensing. While the above sensing applications rely on external interaction with the composites, a more flexible approach is the intrinsic sensing using the inner filter effect [76, 77].

One of the most interesting example of exploiting the inner filter effect was given by [12] where the polymer composites were used to yield the microscopic z-height measuring sensor where the height of the sensor can be measured simple by measuring the red-shift in emission wavelength. This was demonstrated to measure small movements on a chicken lung during using optical measurements.

4.3 Photovoltaics

N. C. Greenham reported photovoltaic effect in a CdSe—MEH-PPV (poly(2-methoxy, 5-(2'-ethyl)-hexyloxy-p-phenylenevinylene) QD-polymer composite [78]**.** Interestingly here the polymer matrix is not just inter-spacer as we saw in the LED applications, but rather MEH-PPV acts as a hole conducting polymer which takes away the photogenerated holes away from the semiconducting QD. This was among the first reports of QD photovoltaics using such conducting polymer matrix, and such photovoltaics are called bulk heterojunction photovoltaics [79]. Further reports from the same group soon after used nanorods like wurtzite CdSe nanodots in poly(3-hexylthiophene) matrix and reported much higher yields [80, 81]**.** It was suggested that the rod-like shape might improve the hole conductance across the QD-polymer junction, hence resulting in higher efficiency of the photo-voltaic. Different iterations of it include using an additional TiO_2 layer for electron transport, which increases the efficiency even further [82]. Other alternative approach developed was to use electron conducting polymer matrix instead.

Following the above discoveries more optically active polymer matrix was considered and it was reported that narrow bandgap polymer, poly[{2,5-bis(2-hexyldecyl)-2,3,5,6-tetrahydro-3,6-dioxopyrrolo[3,4-c]pyrrole-1,4-diyl}-*alt*-{[2,2′-(1,4-phenylene)bis-thiophene]-5,5′-diyl}], (PDPPTPT), when mixed with PbS can be used to yield much more efficient solar cells [83]. Several other similar small band-gap polymers have been since reported PDTPBT [84], P3HT [84], PDTPQx [85] and si-PCPDTBT [86] to work excellently with the quantum dots. It was reported that instead of bulk-heterojunction, if polymer-QD interaction was mediated by a small far-IR active molecule, the efficiency of the solar cell can be improved even further [79].

As an alternative to polymer matrix or small molecule junction, surface functionalized CdSe QDs were also studied, where the surface of the QD was functionalized

using extremely bulky molecule, such as C_{70}. It provides the right balance between bulk-heterojunction and small molecule interface [87].

4.4 Catalysis

The fact that quantum dots are such an efficient photoactive material, is not only exploited in photovoltaics, but also in photo-degradation/photo-catalysis as well. One of the most widely sought after application is the photo-active water splitting. Several reports exist which demonstrate the capability of the QD composites in splitting water and generating hydrogen or simply as a possible fuel cell, e. g. [88] reported a B-doped graphene QD composite for Zn-air battery, and water splitting application. CdS/polymeric g-C_3N_4 composites were reported to have hydrogen evolution rate of about 4.494 mmol h^{-1} g^{-1} under irradiation of simple 300 W xenon lamp [89]. However it seems the majority of community prefers QD-Nanostructure composites over polymer composites for water splitting [90]. Which is understandable as majority of polymeric composites does not favor high water miscibility.

Other than water splitting another emerging use of QD composite photoactivity seems to be emerging in the form of water purification. Under irradiation QD composites can degrade small molecules. Reports of photodegradation of Rhodamine B [91], Methylene blue [92], Ciprofloxacin [93], napthelene and pyrene [94] etc. are aplenty [95].

New exciting application of QD catalysed reactions include polymerization using CdSe QD composite as an photoelectron transfer agent for initiating and catalysing electron transfer reversible addition—fragmentation chain transfer catalysis [7].

4.5 Biomedical

Owing to high quantum yield and narrow excitation spectra of the QDs they have already found strong footing in microscopy fluorescent tags. Gao et. al. [96] used amphiphilic triblock copolymer (hydrophilic polymethacrylic segments, and two hydrophobic polybutylacrylate and polyethylacrylate segments) with TOPO protected CdSe/ZnO coreshell quantum dots. They reported enhanced cancer cell targeting in live mice. They also used prostate binding antibodies, tagged with above quantum dots for active targeting of the cancer cells. A PVP coated BiS QD were used as CT scan enhancing aid. $CsPbBr_3$ Perovskite Quantum Dots with polystyrene-*block*-poly(ethylene-*ran*-butylene)-*block*-polystyrene (PS-PEB-PS) and poly(ethylene glycol)-*block*-poly(propylene glycol)-*block*-poly(ethylene glycol (PEG-PPG-PEG) polymer were used for tracking triple-negative MDA-MB-231 breast tumor-derived exosomes [97]. PEG/CdSe composites were also used for nontoxic live imaging of a Xenopus (frog) embryo [98]. Similar applications have been reported in all aspects of qualitative and quantitative biological imaging.

QD nanocomposites are also making huge inroads in bioimaging and targeted photodynamic therapy. QD nanocomposites are also used as an active agent in photodynamic therapies in mice [99], as an anti-bacterial agent for agricultural applications [100], and very recently as theragnostic probe for COVID detection [101, 102]. PEGylated MoS_2 QDs (MoS_2-PEG) were used for their pH responsive properties and developed a composite that could be used to deliver anti-cancer drug such as doxorubicin to U251 cell lines [103]. Hu et al. developed branched polyethyleneimine (bPEI) based multifunctional photoluminescent carbon dots materials that had quantum efficiencies as high as 54.3%, and were used for gene delivery applications in MCF7 cells [104]. Abdullah-Al-Nahain et al. prepared dopamine hydrochloride conjugated hyaluronic acid (HA), anchored to a graphene quantum dot (GQD) and injected it to tumour-bearing balb/c female mice having overexpressed CD44 receptor [105]. The tumour tissue showed bright fluorescence. These biocompatible materials were successfully sued for drug delivery and in vivo cellular imaging.

There have also been reports of completely novel applications of QD composites, e. g. $Cd_xZn_{1-x}S/ZnS$ core–shell QD—polymer composites can be used as a scintillation gamma ray detectors 5, use of carbon dots in tracing counterfeit currency [106], and as a therapeutic agent in neuro-degenerative diseases [107]. This shows that QD nanocomposites might still have undiscovered applications.

5 Conclusion

In can be concluded that QD and polymer exhibits synergistic effects, enhancing the strength and properties of both via composite formation. Different synthetic routes exhibited various functionalised and well-dispersed QD@polymer composites, among which grafting and template synthesis being the most used. Wrapping technique can be adapted for non-covalent QD-polymer interaction. Uniform QD dispersion can also be achieved using simple bulky ligands to enable steric repulsion which inhibits aggregation. For high photo-luminescent composite fabrication using dewetting can be done to increase surface irregularity, thereby increasing the scattering.

These physical properties of QD-Polymer nanocomposites, when coupled with the optical properties of the QD, results in materials which can be applied to problems ranging from LEDs and white light generation, to bioimaging and photodynamic therapy. QD composites have already made inroads in higher contrast commercial LED displays, and optical microscopy tags. They also seem to be promising in the field of photovoltaics, photocatalysis, water purification, hydrogen/oxygen generation, and chemical sensing.

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Quantum Dots@Metal–Organic Frameworks Composites

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Abstract The development and design of novel quantum dots@metal organic frameworks (QDs@MOFs) composites have been performed to address various societal issues such as energy crises, bacterial infections, the sensing of harmful gases or substances, etc. Combining the advantages of tunable characteristics, unique structural features, and advanced physiochemical properties of MOFs with quantum confinement in quantum dots, these hybrid materials can become a potential candidate for gas storage, catalysis, chemical sensing, and electrochemical energy storage applications. However, MOFs alone possess certain limitations, such as limited electrical conductivity, fixed pore size, and poor stability. These limitations can be overcome by the smart integration of QDs into MOF structures. The resulted host-guest structure shows a wide range of synergetic properties as the host (MOFs) framework suggests appropriate associations to the guest (QDs) for increasing stability, long carrier lifetime, enhanced, higher photoluminescence quantum yield and synergic properties. Thus, this chapter delivers a perception about the recent breakthroughs in the field of QDs@MOFs, their historical evolution, structural features, various synthesis strategies, and progress towards their utilization in numerous applications, including optoelectronics, biomedical, electrocatalysis, and energy storage. Along with this, it also addresses conclusion and future prospects of QDs@MOFs hybrid materials towards their implementation in energy and environmental applications.

Keywords Quantum dots · Composites · Photoluminescence · Metal–organic frameworks · Catalysis

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List of abbreviations

QDs@MOFs	Quantum dots@metal organic frameworks
ZIF	Zeolite imidazole frameworks
LEDs	Light emitting diodes
PeQDs	Perovskite QDs
$Ca(OH)_2$	Calcium hydroxide
PbO_2	Lead oxide
PbS	Lead sulfide
CdS	Cadmium sulfide
CdSe	Cadmium selenide
Cos	Composites
MBE	Molecular-beam epitaxy
CdTe	Cadmium telluride
GQDs	Graphene quantum dots
HRTEM	High-resolution transmission electron microscopy
BTC	1,3,5-Benzenetricarboxylic acid
TiO_2	Titanium dioxide
ZnO	Zinc oxide
$Pb(Ac)_2$	Lead acetate
HPLC	High performance liquid chromatography
DA	Dopamine
CDs	Carbon dots
BPA	Bisphenol A
CQDs	Carbon quantum dots
ECL	Electrochemiluminescence
TPA	Terephthalic acid
NiS	Nickel sulfide
AFM1	Aflatoxin M1
MoS_2	Molybdenum disulfide
CCQDs	Chiral carbon QDs
TDPAT	2,4,6-Tris (3,5-dicarboxylphenylamino)-1,3,5-triazine
DNA	Deoxyribonucleic acid

1 Introduction

Metal-organic frameworks (MOFs) are enthralling materials which exhibit adjustable surface functionalities, tunable porosity, and their usefulness in hybrid or composite structural composition based applications [1]. These functional materials are designed by a combination of definite metal nodes with organic linkers (Fig. 1), resulting in materials with tunable pore size, an overall framework, structural stability, and expanded physicochemical properties [2–5]. Thus, this results in their utilization

in various applications, for example, electrocatalysis, gas storage or separation, drug delivery, chemical and physical sensing, etc. [6]. Among these, metal ions with coordinatively unsaturated active sites and luminescent features in the counter parts of MOFs such as MIL, UiO, ZIF (zeolite imidazole frameworks), PCN, HKUST (Cu-MOF), and IR-MOF, etc. behave as functionally dynamic sites for selective sensing applications [7, 8]. Sustainability in the pores of MOFs acts as an ordinary territory for guest molecules, which results in improving sensing properties due to their better interaction with guest molecules [9]. These rigid MOFs exhibit higher thermal stability with durable structural support for fluorescent guests. Due to the reduction in the non-radiative easing of organic linkers in MOFs, the origin of strong fluorescence emission has received substantial attention for various applications. Most recently, there have been some reports of buckling of the overall straight structure of MOFs due to the uniaxial pressure on them. Resulting MOFs show quantum behaviour at lower temperature (T) due to the superposition of degenerated bucking states and open a new pathway towards strain caused quantum states. At T = 0 °C, three strained buckling phases (normal, parabuckling, and ferrobuckling) are observed in MOFs due to the quantum phase transitions [10].

Despite being so advantageous, there are certain limitations to MOFs that hinder their direct utilization in various applications. These can be overcome by uniting them with other materials such as highly conductive polymers, quantum dots (QDs) or other semiconductive materials to enhance their properties. Thus, QDs are classified as a class of nanomaterials that exhibit three dimensional confinement, i.e., classified as zero dimensional (0D) particles with dimensions shorter than the Bohr exciton radius [11]. These are generally synthesized using materials from elements of groups I–III–VI [12], II–VI [13], III–V [14–16], and IV [17] and exhibit various properties with disparities in their sizes ranging from 2 to 10 nm [18]. Based on their bandgaps, the materials emit a wide range of colors upon absorption of white light [19, 20]. As a result, these materials find applications in photovoltaics, light emitting diodes (LEDs), energy storage, and targeted drug delivery, etc. [17, 21, 22]. QDs (size < 10 nm), exhibit quantum effects including inelastic electron tunnelling, quantum tunnelling, Fermi-Dirac distribution, etc. [23]. However, these materials also exhibit certain limitations, such as the easy agglomeration pertaining to fluorescence quenching, which limits their utility in a various of applications. Significant efforts have been invested to overcome the hurdle of passivation by utilizing supplementary semiconductor films with an appropriate bandgap and embedding QDs inside a wide range of materials (e.g., organic polymers or micelles, etc.). Though all the reported methods are time consuming and can lead to undesirable products with unexpected behaviour and properties. Thus, it has been attempted to encapsulate the semiconductor nanomaterials with MOFs to get QDs with multifunctional functionality and adaptable composite architectures [24]. Incarceration of purely inorganic perovskite QDs (PeQDs) embedded inside MOFs e.g., UiO-67-4,4′-bipyridyl and MIL-101(Cr) in order to synthesize PeQDs@MOFs provides a wide range of advantages such as electronic structure, band gap tuning, thermal stability, chemical reactivity, and ultrafast scintillator application [25, 26]. The aim of this chapter is to discuss the synthetic approaches for QDs@MOFs composites

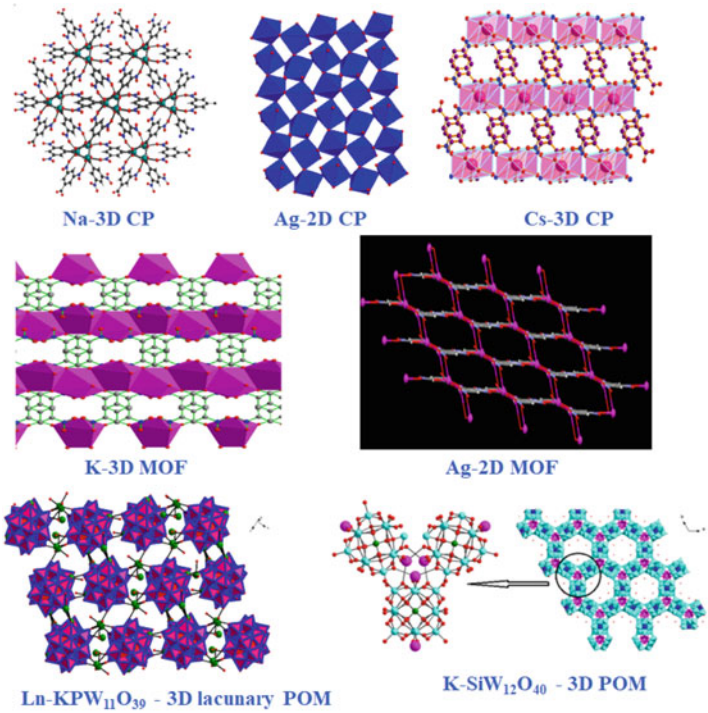

Fig. 1 Examples of earlier reported coordination polymers (Na-3D CP, Ag-2D CP), MOFs (K-3D MOF and Ag-2D MOF) and Polyoxometalates (Ln-$KPW_{11}O_{39}$—3D lacunary POM, K-$SiW_{12}O_{40}$—3D POM), where CP = coordination polymer, POM = polyoxometalates, reproduced with permission from the References [3–5]

and their advancements over MOFs and QDs. Additionally, the discussion related to the structural properties, activity, and selectivity of composites towards various catalytic reactions and other applications have been discussed in details.

2 Historical Evaluation of MOFs, QDs and Composites (Cos)

According to literature, the fields of zeolites, solid state, and coordination chemistry came into reality before the origin of MOFs. Thus, there is a wide history related to the investigation of coordination compounds for numerous applications. Since 1964, curiosity about porous materials such as Prussian blue and Hofmann has increased due to their adjustable adsorption properties [27]. Subsequently, in early 1990s, the first report about MOFs was published followed by more than twenty thousand examples of MOFs have been reported in the last thirty years. These materials are

important due to their extreme porosity with 90% free volume inside the framework and broad internal specific surface areas (more than 6000 m^2/g) [28]. Hoskins and Robson reported basic information related to the future benefits of MOFs in their articles from 1989 to 1990. After their findings, many researchers all over the world have published reports related to the construction of an extensive range of highly ordered crystalline compounds, conceivably utilizing structure-templating agents and post-synthetic modification approaches. Yaghi et al. in 1995 [29] first coined the popular term MOF. In 1997, Kitagawa and co-workers reported a 3D MOF that shows excellent gas sorption properties [30]. Later, the MOF-518 and HKUST-119 came into the attention in year 1999 and in 2002, Férey et al. reported the permeable and flexible MOFs (MIL-47, MIL-53, and MIL-88) [31]. In continuation of the progress in MOFs, the idea of isoreticular chemistry originated in 2002 for simple Zn dicarboxylates and mixed-ligand based MOFs and then spread to other compounds. In the same year, the MOFs family has expanded to include imidazolate-based compounds, currently known as zeolite imidazole frameworks (ZIFs). Apart from the ZIF family, plenty of examples of MOFs, including UiO-66 (Zr), MIL-125 (Ti), MIL-101 (Cr), MIL-100 (Fe), and, CAU-1 (Al) etc. have been reported by researchers along with their usefulness in various applications [32].

In addition to this, the history of QDs is over 2000 years old; where naturally occurring, cost effective substances such as calcium hydroxide ($Ca(OH)_2$), lead oxide (PbO_2), and water were utilized for the synthesis of lead sulfide (PbS) QDs [33]. Then, these were utilized by Roman and Greek civilizations for applications such as hair dyes [34]. Later in the 20th century, Cadmium sulfide (CdS) and Cadmium selenide (CdSe) QDs were utilized in the preparation of the colourful glasses by controlling their size in silicate glasses. Such doped glasses found their application in optical filters in 1932 Following this from 1991, great deal of interest moved towards establishing the synthesis approach towards the growth of QDs. Thus, this two—era's of history of QDs, ranging from ancient hair dye applications to scientific advancements, shows an increasing trend in the applications of these materials from lab to their commercialization [34]. Depending on the QDs synthesis techniques, the two major classes are categorized as *top-down* and *bottom-up* techniques. *Top-down* technique includes the division of the main material into smaller subunits by using various processes (e.g., ion implantation, and X-ray lithography etc.) [35] and is also known as thinning down technique. Whereas the *bottom-up* technique results in the growth of QDs from the precursor solution. This is the most utilized technique, which includes the self-assembly of the QDs in the solution over time [36, 37]. Depending on the growth conditions, bottom-up techniques include two major synthesis methods: (a) wet chemical method, (b) vapor phase method. As the name suggests, *wet chemical* technique involves the utilization of the precipitation technique for the synthesis of QDs. Depending on solute, solvent, and growth conditions, nucleation can be classified as homogeneous and heterogeneous nucleation. Homogeneous nucleation on one side arises from the alliance of the solute atoms/molecules and reaches to a critical size without any solid interface. This includes techniques such as sol-gel, hot solution decomposition, microwave, and microemulsion techniques [38]. Major advantage of this technique includes the lower synthesis cost,

along with challenges such as toxicity to humans and environment. Thus *biogenic approach* which is the extended version of the wet technique and offers advantages in terms of bio-compatibility and lower toxicity [39, 40]. The vapor phase method, also known as the physical approach, in the synthesis of QDs involves the atom-by-atom deposition of the QDs using techniques such as molecular-beam epitaxy (MBE), sputtering, etc. This process of nucleation of particles in the vapor phase to form QDs results in advantages such as simple and controlled fabrication technique, but a major challenge is the higher cost.

Along with advantages, QDs suffer from various challenges, including agglomeration. Thus, for better utilization of their properties, they are generally used in combination with other materials, such as composites (Cos). Cos are types of materials that are designed such that the advantages of both components can be effectively utilized, avoiding the shortcoming of both [23, 41]. QD@MOF Cos were first introduced in 2010 by Feng et al. [42], where they utilized ZnO QDs in combination with the MOF-5. In this report, the main emphasis was on the evaluation of the optical properties of Cos. Additionally, many other reports published in the same year 2010 which were later described by Lopamudra Giri et al. [24] in 2022. In this study, a wide range of synthesis techniques, i.e., bottle-ship, ship-bottle, photochemical deposition, sol-gel, template assisted method, drop casting, in-situ consecutive chemical bath deposition, physical fusion, photodecomposition, direct deposition, spray coating, simple dispersion, pyrolysis, calcination, direct surface functionalization, intercalation, co-electrospinning followed by carbonization and hydrothermal treatment, thermal induction, bi-sacrificial templates etc. have been discussed [43]. On the other side, highly porous MOFs with a large surface area deliver a thrilling podium to create a superlative atmosphere for QDs loading and prevent the chances of aggregation. Simultaneously, QDs develop the physicochemical behaviour of MOF materials. The combination of both QDs and MOFs results in good dispersibility, resulting in overall stability. In this situation, QDs@MOFs Cos have piqued curiosity and then unlocked an advanced avenue for various applications These hybrid substances have captivating properties, such as good mechanical or thermal stability, outstanding photoluminescence, excellent biocompatibility, and simplicity of functionalization [26]. A list of advantages offered by the QD@MOF Cos includes:

- *Improved stability*: The MOF matrix protects the QDs from degradation due to environmental factors including moisture, temperature, and oxidation, leading to their higher stability.
- *Tunable porosity*: The porosity of the MOF can be controlled as per the QDs used for better stability of the QDs in the matrix. This also improves the performance of the Cos for applications such as sensing, catalysis, etc.
- *Controlled interfaces*: The interface between QD and MOF can be precisely controlled, leading to controlled charge transfer processes between both QD and MOFs.

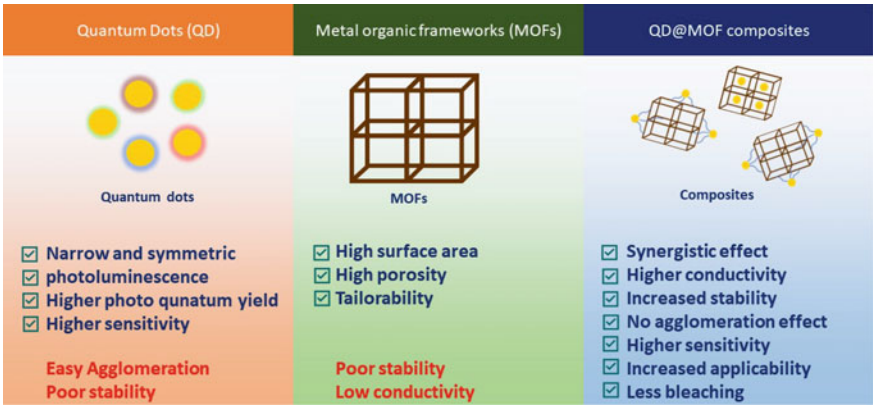

Fig. 2 Illustration of the characteristics of the QDs, MOFs and their composites

- *Enhanced photoluminescence (PL)*: The integration of QD and MOF results in increment in light emission, with only QDs showing improvement in light emitting efficiency by incorporation of both.
- *Improved properties*: *S*tandalone QDs are mostly utilized for LEDs, and light absorption applications, while MOFs generally offer traits such as porosity, functionality, etc. Thus, combining both as Cos results in more versatile applications than standalone.

Thus, these enhanced features by combining both MOFs and QDs open pathways for more advanced possibilities in photonics, sensing, and catalysis applications. Figure 2 illustrates the characteristics of QDs, MOFs and their composites; indicating that combination of both can bring new possibilities due to their synergetic effects. Thus, the goal of this book chapter is to take a deep dive into the world of QD-MOF Cos, their synthesis techniques, material selection depending on application.

3 Synthesis Techniques of QD@MOF Cos

As stated above there are various reported techniques for the fabrication of the QD@MOF Cos. For the synthesis of Cos pre-synthesized QDs and MOFs are utilized in various ways as shown in Fig. 3 depending on the MOFs, QDs properties and required applications.

Initially, photochemical deposition and direct surface functionalisation of semiconductor QDs with MOFs were the two major techniques utilised for the preparation of the QDs@MOFs Cos [44, 45]. The construction of QD@MOF Cos by photochemical deposition requires two significant strategies, which are *in-situ* development of QDs and their accumulation on the exterior sides of MOFs based on proper photoreduction potential. Direct surface functionalization includes the immobilization of

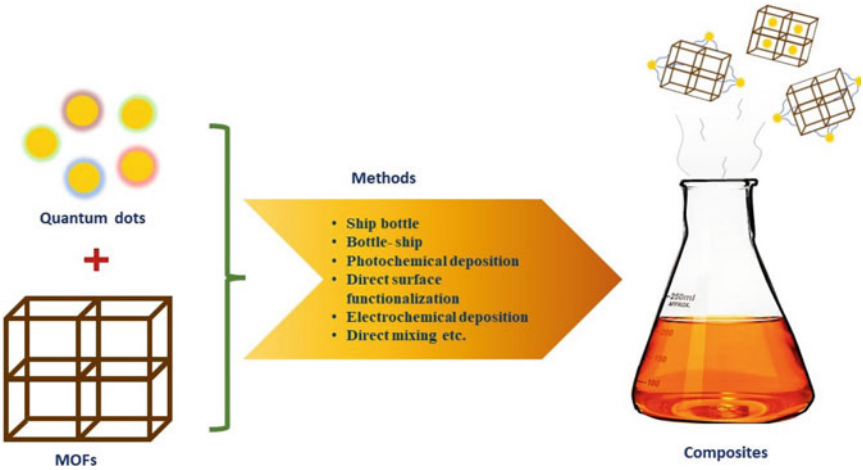

Fig. 3 Methods of the production of QD@MOF Cos [43]

QDs on MOFs by suitable ligands. There are different capping agents used to change the surface of QDs and directly make contact with respective MOFs by coordinating the association between the overlaying agents of QDs and the centre metal ions of MOFs. This synthetic approach has some advantages in that the morphology of MOFs; with proper controlled shape and size QDs.

In addition to these two methods, the fabrication of ZIF-8@CQDs can be done by in-situ fabrication involving the direct mixing of all precursors at room temperature [46]. It has been observed that the resulting ZIF-8@CQDs composite is an example of perfect luminescent MOFs-derived sensory materials because high quantum yields containing luminescent guest materials have been introduced inside the MOF channels.

There is other two well-constructed approaches i.e., '*ship-bottle*' and '*bottle-ship*' which find application of QDs inside- outside of MOFs pores. Among these approaches, the *ship-bottle* method as the name suggests focuses on fitting of the QDs into the pores of the MOFs. This immobilisation of small nanoparticle precursors/QDs into pores leaves less tendency for them to pierce through the pores of MOF, followed by further treatments to transform them into the required practical assembly. For the synthesis of QD@MOF Cos, the QDs precursors are loaded into the MOF by heating, hydrogenation, and reduction reactions [44]. Consequently, in the *bottle-ship* method, the QDs@MOFs Cos encloses the guest material (QDs) with QD size > MOF pore size; retaining it outside the lattice structure. There have been numerous reports showing the applicability of the ship-bottle approach, i.e., H. Kitagawa and co-workers summarized palladium-based nanocubes with HKUST-1 (an example of Cu^{2+} ion-based MOF) without any obvious organic linker molecules [47]. It has experienced an upsurge in the ability for hydrogen storage through modification of the outer surface of palladium atoms with MOF. Consequently, the formation of direct

contact between MOF and nanoparticles is anticipated to become fascinating for QDs and their luminescent properties are vulnerable to the surface conditions. It has been explored the prospect of employing MOFs as novel classes of surface shielding materials for fluorescent QDs. ZIF-8 MOF utilized to cover CdSe/CdS core/shell QDs via a direct connection between the two species in the absence of other bonding constituents. They have aimed to synthesize Cos which would bring the cooperative effects of both tunable MOFs and semiconductor QDs. Oleylamine of the QDs has switched with pyridine, and then the pyridine-plugged QDs undergo in-situ ligand exchange with 2-methylimidazole of ZIF-8. Further using a controlled concentration of ZIF-8, monodispersed growth of ZIF-8 on the outer layer of CdSe/CdS core/shell QDs is obtained. Fully merged QDs into the ZIF-8 framework show excellent photoluminescence properties, with photoluminescence quantum yield >40% as compared to the untreated QDs [45]. The major obstacle to the utilization of the ship-bottle technique is the lack of control over the inner surface, shape, and size of guest molecules within the MOF structure [48]. Thus, ship-bottle method could be disadvantageous for applications where the shape of QDs is predominantly important; i.e., utilization of the gold nanoparticles for biomedical applications. On the flip side, for the bottle-ship method, a stable framework is required under optimised conditions to construct the functional material or nanoparticles on the outer part of MOFs.

4 Structural Features and Properties of MOFs@QDs Cos

It has been proven from literature that the methods of preparation for the MOFs@QDs Cos play crucial roles in controlling their physical properties for various applications. In earlier reports, the injection reaction protocol was utilised for the synthesis of ultra-small (~2 nm) CdSe QDs in the confined cavities of MIL-101(Al)-NH_2 [49]. Due to the mismatch in the size of QDs and the pore size of MOF, the morphologies of the resultant QD@MOF Cos can be varied and are categorised into three sub categories. In the former case, QDs are completely encapsulated by the frameworks of MOFs. The evolution and orientation of QDs are limited or negligible. So, the QDs cannot be mobilized in the MOF matrix in any order to get agglomerated. For the fabrication of luminescent semiconductor QDs, MOFs can act as efficient stabilizing agents because of their insulating nature and sometimes semiconducting properties, as well as the versatility of the designed materials [45]. In most of the QD@MOF Cos, there are some direct bonding interactions between the QD and MOF-like polymers, which are required to exploit the synergistic effects of both QDs and MOFs. A dropwise mixing of MOFs based solution into QDs solution, an easy detachment of pyridine derivatives, and the growth of MOFs on the outer surface of QDs has been observed. The resultant composite has been characterized based on photoluminescence spectra. The complete shielding of MOFs on the outer layer of QD has established by the impassiveness of the QDs@MOFs to the solid-state photoluminescence quenching tests [45]. In an earlier report, the construction of the

core-shell assembly of CdTe@ZIF-8 (CdTe = Cadmium telluride) was mentioned, in which various numbers of CdTe QDs were orderly dispersed in the ZIF-8 framework [50].

Additionally, the encapsulation of graphene quantum dots (GQDs) inside the pores of ZIF-8 framework shows the robust connections between Zn^{2+} ions of ZIF-8 and −COOH groups of GQDs [51]. Such interactions are forming zinc-carboxyl bonds which have endorsed the association of GQDs and ZIF-8 and withdrawn the chances of GQDs aggregation in ZIF-8. In the second case, some QDs are surrounded by the MOF matrix, although others are unprotected from the MOF's surface. The probable cause is that some QDs are larger in size than the pores of MOFs, and sometimes the amount of QDs is high as compared to the voids of MOFs. Consequently, all the QDs cannot enter the pores of MOFs and some QDs are exposed on the surface of MOF. He et al. captured CdS QDs inside the pores of MIL-101, where the excess CdS QDs had aggregated on the surface of MIL-101 [52]. Similarly, Gan et al. fabricated the CdSe QDs@UiO-66 in which the CdSe QDs have been encapsulated by the UiO-66 framework [53]. It has been shown from high-resolution transmission electron microscopy (HRTEM) images that CdSe QDs have grown both internal and external surfaces of the UiO-66. The particle size of CdSe QDs on the outer surface was bigger than those inside the MOFs. The third case shows that QDs are affixed to the external surface of MOFs, so their growth is not constrained and leads to excessive accumulation on the MOFs. This concept is supported by the earlier report of Li and co-workers, in which they fabricated $Cu_3(BTC)_2$ (BTC = 1,3,5-benzenetricarboxylic acid) with a core-shell structure. The titanium dioxide (TiO_2) shell with a thickness ~210 nm has bounded the central portion to form a porous and rough texture on $Cu_3(BTC)_2$ [54]. Similarly, Xu et al. have immobilized ZnO (zinc oxide) QDs with ~10 nm particle size on the UiO-66 (Zr-MOF) with a fixed pore size the MOF. Consequently, the ZnO QDs did not enter the Zr-MOF matrix but rather agglomerated on the surface of Zr-MOF, which is established by the TEM images [55]. Direct impregnation of GQDs with ~3.1 nm particle size into a mesoporous porphyrin Zr-MOF has increased electrical conductivity and electrochemical sensing activity by simply transferring charge from GQDs to porphyrin linkers of Zr-MOF. The resultant composite holds half the porosity compared to the parent MOF and contains 100-fold advanced electrical conductivity. The utilization of porphyrin linkers in Zr-MOF as highly catalytic active components for the synthesized GQD@MOF composite to display improved electrochemical sensing activities towards nitrite in aqueous medium compared to both GQD and the parent MOF [56]. The in-situ confined progress of ultrasmall PeQDs in the UiO-66 MOF matrix has performed to get their quantum confinement effect. These composite materials display gifted optical and optoelectronic properties for photovoltaic applications by regulating the particle size from 6.4 to 3.3 nm with variation in the concentration of lead acetate ($Pb(Ac)_2$). The reduction in the size of PeQDs results in a blue shift in their PL emission spectra from 521 to 486 nm due to the enhanced quantum confinement effect. Thus, the UiO-66 matrix has endowed the surface passivation effect, and ultrasmall PeQDs have exhibited a 43.3% photoluminescence quantum yield with a large photoluminescence lifetime of 100 ns [57]. In another case, $AgInS_2/GaS_x$ core/shell QDs encapsulated

by indium-fumarate MOFs led to an enhancement in structure stability with a slight transition in band-edge and a 12.6% photoluminescence quantum yield; which will be conserved after one week due to the enhanced stability of encapsulated QDs in MOF [57].

5 Applications of QDs@MOFs Cos

After the comparison of previously reported porous inorganic substances with the MOFs based composite, it has been proposed that there is diversity in the chemical compositions of MOFs, which allows a wide range of structure features and functionalities to be tuned by using different building blocks. These Cos exhibit features such as ultrahigh specific surface areas, easy tunability, and micro-porosity, resulting in enhanced structural properties leading to their utilization in various applications (e.g., photocatalysis, gas storage, sensing, energy storage, targeted drug delivery, and antibacterial activities) [44]. Thus, this section of the chapter primarily recapitulates various applications of QD@MOFs in detail. Moreover, it also describes the opportunities related to the QDs@MOFs Cos and briefly discusses various challenges associated with Cos during synthesis along with their stability issues.

5.1 Sensing

Luminescence based sensing is one of the major breakthroughs in sensing applications using QD@MOFs [58] because of the higher quantum yield achieved by integrating QDs and MOFs. A broad range of sensing applications using these materials include Cu^{2+} ion sensing, visual detection of glucose, dopamine detection, etc., where these materials are utilized as fluorescent sensing probes or electrodes. Along with this, these Cos offer a wide range of advantages, such as a facile or cost-effective synthetic approach, environmental friendliness, ultrahigh PL stability, lower toxicity, and excellent biocompatibility. Various sensing applications can be further described as follows.

5.2 Detection of Dopamine

Dopamine (DA) is a type of neurotransmitter in the human central nervous system that contains catecholamine. It generally takes part in numerous biological activities, such as central nervous system, hormonal, and cardiovascular system. The deficiency of dopamine may lead to poor regulation of muscular movement, resulting in the distraction of muscles and leading to Parkinson's disease. To date, plentiful methods have been developed by chemists to regulate the level of dopamine, including UV

spectrophotometer, electrochemical analysis, high performance liquid chromatography (HPLC) and various fluorescent probes. However, these methods have some shortcomings, such as lethal substances and organic ligands, etc., which would confine their applications, difficult instrumental techniques, and complicated sample pre-treatment procedures. To overcome these difficulties, it is extremely required to grow eco-friendly, facile, and commercial approaches to regulate the amount of dopamine. However, there are limited reports for determining the quantity of dopamine by utilizing carbon dots (CDs)@MOFs Cos. In one of the reports, it has been observed that CDs with high quantum yield have been embedded within the voids of ZIF-8 matric for the facile assembly of CDs@MOFs (Fig. 4) which can behave as fluorescent probes for smooth recognition of the dopamine concentration. In CDs@MOFs, the synergistic effect between CDs and MOF matrix shows remarkable results. It has provided a habitual situation to sensibly capture dopamine into the MOF pores and harvest the bonding interactions between dopamine and the basic spot of ZIF-8 (Fig. 4a). Dopamine has been effortlessly oxidised to harvest dopamine-quinone and captivated on ZIF-8 by the weak π···π interactions with phenyl rings of ZIF-8 MOF, resulting in fluorescent quenching of CDs (Fig. 4b). CDs have been utilized as fluorescent signal probes to transmute chemical signals of contacts between CDs and dopamine. The sensing performance of CDs for dopamine is uniformly improved compared to earlier reported signals, with a wide range of concentration (0.1–200 μM) and a low detection limit of 16.64 nM [46].

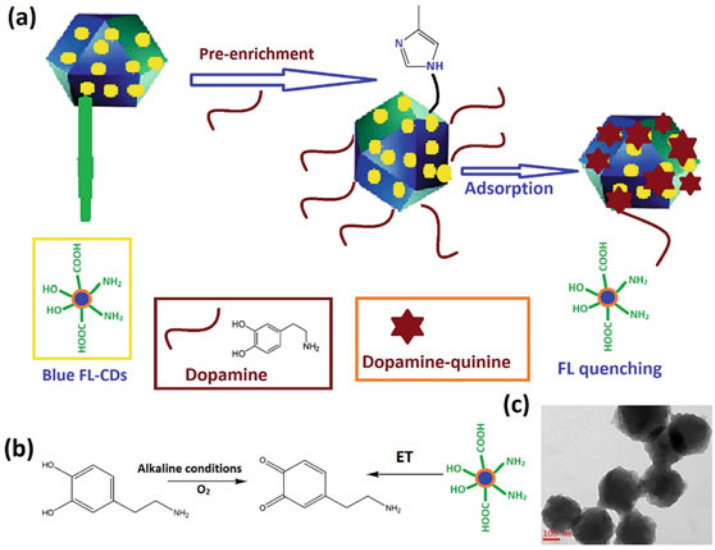

Fig. 4 **a** Schematic representation of the CDs@ZIF-8 synthesis and their application in the recognition of dopamine; **b** possible mechanism for the detection of dopamine; **c** HRTEM image of the CDs@ZIF-8/DA-quinone, reproduced with permission from the reference [46]

5.3 Sensing of Endocrine-Disrupting Chemicals

There are a series of dangerous endocrine-disrupting chemical substances (e.g., dioxins, triethyl lead, bisphenol A, vinclozolin, and phthalates etc.) that may result in numerous health issues, especially for the reproductive systems and transgenerational diseases in adults. From them, bisphenol A (BPA) is known as the endocrine-disrupting substance. It is a predominant substance due to its use as a raw material for manufacturing rigid foams, thermoplastic organic polymers, and large-scale industrial coatings. Therefore, there is a need to grow sensors or probes for the initial discovery of endocrine-disrupting chemicals [59].

5.4 Detection of Ascorbic Acid

Lately, a dual-emission ratiometric sensor such as CdTe QDs@UiO-66-NH_2 has been constructed and used for the sensing of ascorbic acid [60]. Ascorbic acid is a vital micronutrient and antioxidant that is used by various biosynthetic enzymes in several physiological and pathological processes. Ascorbic acid can also be oxidized to synthesize dehydroascorbic acid and remove oxidative stress from living organisms. The amount of ascorbic acid greater than 110 μM, creates major health issues such as diarrhoea, urinary stones, and stomach cramps. Consequently, quantitative analysis of ascorbic acid is the key method for primary recognition of associated health diseases. Lately, fluorescence sensing has attracted major attention to determine the concentration of ascorbic acid because of its rapid response time and better sensitivity. Though, in earlier reports, the fluorescent probes provided only a single emission feature and were blocked by numerous interferences, including instrumental parameters, environmental response, photobleaching, and the concentration around probes or sensors. Therefore, a stable fluorescent sensor, including both CdTe QDs and UiO-66-NH_2 MOF is required to detect the amount of ascorbic acid and the pristine MOF displays only a single emission peak. The simple mixing of MOF, i.e., UiO-66-NH_2 and CdTe QDs, includes the electron transfer from CdTe QDs to MOF UiO-66 (Fig. 5), resulting in an intrinsic dual emission peak [60]. Besides, the fluorescence intensity of CdTe QDs@UiO-66-NH_2 MOF can be transformed by ascorbic acid, the emission band of UiO66-NH_2 MOF is reduced, though the emission band intensity of CdTe QD is amplified after adding ascorbic acid. In conclusion, the CdTe@UiO-66-NH_2 behaves as an excellent fluorescence sensor to show virtuous sensitivity towards ascorbic acid.

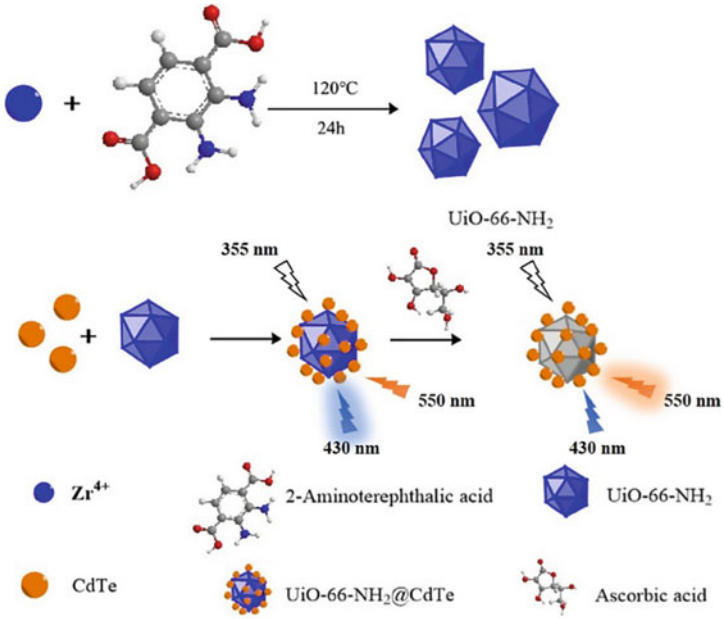

Fig. 5 Diagrammatic representation of the construction of UiO-66-NH_2@CdTe, reproduced with permission from the reference [60]

5.5 Highly Sensitive Detection of 4-nitrophenol

For the manufacturing of dyes, pharmaceuticals, and leather products, 4-nitrophenol is known as a common intermediate that comes out in all reactions. Nevertheless, 4-nitrophenol is also predicted to be a common lethal toxin that causes extreme harm to the human health and creates many environmental issues. Therefore, selective detection of 4-nitrophenol is of prodigious importance to save human health and protect the environment. Smartly impregnating CQDs (carbon quantum dots) within the voids of Zr-MOFs leads to the formation of fluorescence-functionalized Cos by applying a post-synthetic alteration approach. The resultant CQDs@Zr-MOF act as fluorescent probes for the determination of toxic substances [61]. Hence, a rapid, facile, and relative sensitive approach has been developed to synthesize NH_2-CQDs@UiO-66 MOF fluorescence sensor probe to detect the traces of 4-nitrophenol with 3.5 nM as a low detection limit. In order to expand the selectivity and sensitivity of fluorescence sensors, either UiO-66 MOF has been utilized to enrich the target molecules or fluorescent NH_2-CQDs have been used directly to sense the synergistic effect and interactions between UiO-66 MOF and targeted 4-nitrophenol [62].

5.6 Selective Gossypol Detection

Gossypol is known as a category of polyphenolic toxins that are naturally occurring within the mallow family. This substance is primarily present in the cottonseeds and can be easily removed from cotton-based plants. There is a fear related to the protection of cottonseed plant-based products (e.g., animal feed and cottonseed oil) from gossypol. Major issues related to gossypol are that its excretion from human body is very slow, and ingesting the food items with extreme gossypol accumulates toxic substances and shows adverse health effects [63]. In reports, multiple interpenetrating MOFs with channel-size-dependent behaviour easily encapsulate the PeQDs, resulting in a PeQDs@MOFs composite that is useful for the selective detection of gossypol and in host-guest chemistry [63].

5.7 Targeted Drug Delivery

Conjugated MOFs and QDs based Cos are useful for target drug delivery applications and bioimaging of cancer. All-inorganic PeQDs of $CsPbBr_3$ captured within the voids of ZIF-8 to form $CsPbBr_3$ QD@ZIF-8 MOF Cos by utilizing an in-situ chemical reduction approach. The $CsPbBr_3$ QD@ZIF-8 MOF Cos further tried for an advanced technique, i.e., surface-enhanced Raman scattering, in which a charge transfer is noted within the composite, and indicating the potential application of the composite for surface-enhanced Raman scattering detection. The adsorption behaviour of 4-mercaptopyridine over $CsPbBr_3$ QD@ZIF-8 MOF has been evaluated by utilizing typical surface-enhanced Raman scattering spectral bands [64].

5.8 Energy Storage Applications

In earlier reports, GQDs@MOFs have been used to derive porous carbon for electrode material with enhanced semiconducting behaviour for supercapacitors with ideal electric double layer capacitor (EDLC) behaviour and lithium/sodium-ion batteries [65]. In another report, a self-sustaining suprastructure has been fabricated by sequentially loading NH_2-CDs and $CsPbBr_3$ PeQDs into the hierarchical ZIF-8 for electrochemiluminescence (ECL) bioanalysis [66]. The resultant PeQDs@ZIF-8 MOF composite has enhanced structural stability because ZIF-8 contains a protective layer and enhances charge transfer from CDs to MOFs. There is also an efficiently self-enhanced ECL emitter shown between PeQDs and NH_2-CDs in a compact structure with the confinement effect. Thus, the ternary Cos (NH_2-CDs@PeQDs@ZIF-8) is gifted with excellent stability and better ECL efficiency [64]. For the first time, this ECL probing approach is used on ternary Cos (used as an electrode substrate) to examine the T4 polynucleotide kinase activity [66].

5.9 Electrocatalysis

From earlier reports, utmost MOFs are electrically insulating in nature, which limits their practical utility in electrochemical (Fig. 6) and optoelectronic applications. Though various synthetic or post-synthetic approaches have been established to project electrically conducting MOFs with tunable pores and structure stability [67]. The main example of such modifications is the introduction of numerous guest (QDs) molecules to reduce the charge transfer between donor–acceptor in respective MOFs. The most common approach to reducing the charge transport in Zr-MOFs is to utilize redox hopping under different electrochemical situations. Generally, the slow redox hopping in Zr-MOFs frequently limits the electrochemical activities [68]. In an earlier report of GQD@PCN-222, GQDs have an extra negative conduction band as compared to the porphyrinic linkers of Zr-MOFs. Typically, the charge transfer between donor and acceptor has been recorded from electron-releasing GQDs to electron-accepting porphyrinic linkers, resulting in heightened electrical conductivity and improved electrochemical sensing towards different nitrites [56].

In another report, NiS/Ni_3S_2 QDs@NiFe-terephthalic acid (TPA) nanosheets (~5 nm) were prepared by an *in-situ* two step hydrothermal method and used for water splitting applications [26]. These nanosheets show only 219 mV overpotential (current density = 10 mA/cm^2 for 60 h). With increasing the amount of sulphur-containing agent, the Ni_3S_2@NiFe-TPA displayed excellent HER performance. During complete water electrolysis, the electrolyzer has been accumulated with N/S/QDs@NiFe-TPA and Ni_3S_2@NiFe-TPA as an anodic electrode. The cathodic electrode displays 1.66 V a low cell voltage at 10 mA/cm^2 current density, and retained the structure of Cos after 60 h. The admirable electrocatalytic properties of these Cos are because of their synergistic effect between Nickel sulfide (NiS) and MOFs, which can offer quick ion or electron transportation, reachable active sites, and appropriate O_2 and H_2 release channels.

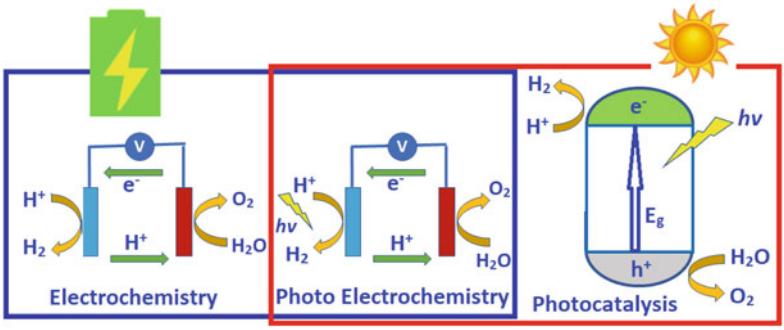

Fig. 6 Schematic representation of mechanisms of electrochemical, photo electrochemical and photocatalytic processes

5.9.1 Electrochemical Biosensor

Molybdenum disulfide (MoS_2) QDs@MOFs Cos can behave as electrochemical biosensors for the recognition of Aflatoxin M1 (AFM1) [69] and their working principle is as shown in Fig. 6. AFM1 are known as hepatotoxic metabolites, which are carcinogenic and root contrary human health issues such as diminished immune response, liver functioning issues, and numerous infections. The electrochemical biosensor for the active recognition of AFM1 has been designed by the modification of screen-printed carbon-based electrodes with MoS_2 QDs and UiO-66-NH_2 and decorated with AFM1. The developed biosensor has been validated according to its performance in recognising AFM1 in spiked milk samples. In another report, a Cu-TDPAT@CCQDs/NiF derived electrode (where, TDPAT = 2,4,6-tris (3,5-dicarboxylphenylamino)-1,3,5-triazine) has been prepared by electrodepositing MOF and chiral carbon QDs (CCQDs) on the nickel foam (NiF). This electrode can be used for the electrochemical enantio-recognition of tyrosine isomers from racemic mixtures with high selectivity and sensitivity [70].

5.10 Antibacterial Activity

New GQDs modified with NH_2-MIL-125 are efficient for antibacterial activity. Bacterial infection threatens human health, and an innovative photocatalyst, GQDs@NH_2-MIL-125 (Fig. 7), has been used for its photocatalytic disinfection and antibacterial properties. Active substances have destroyed the cell membranes and deoxyribonucleic acid (DNA), completely killing the bacterial infection. The chelation in NH_2-MIL-125 MOFs may diminish the polarity of metals, thus increasing the lipophilicity and enabling the NH_2-MIL-125 to breach the wall of a bacterial cell for killing the bacteria [71].

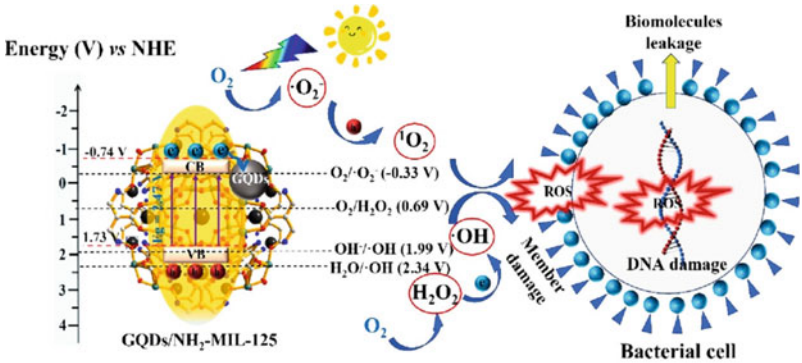

Fig. 7 Proposed mechanism of GQDs/NH_2-MIL-125 (UiO-66 MOF), reproduced with permission from the reference [71]

5.11 Photocatalysis

In current years, environmental pollution and the depletion of energy resources have become major problems for society. Photocatalysis (Fig. 6) is an effective technique to produce energy from renewable sources and is helpful for environmental remediation. Therefore, the emerging field of research is transiting towards the development of novel photocatalysts with their utilization for energy generation and storage. Nevertheless, there are a few drawbacks to single-phase photocatalysts, such as poor tunability, a wide bandgap, easy agglomeration in solution, a higher photogenic charge carrier recombination rate, slow photogenic electron transmission, and responding only to ultraviolet light. These limitations may lead to low photon energy utilization proficiency and low photocatalytic activity. To solve these issues, a combination of MOFs and semiconductor QDs has been reported to make composites that show porosity, exhibit large surface area. In these Cos, QDs are distributed inside the pores to decrease the rate of aggregation and enhance their light interaction capacity. Due to unique structural features of MOFs (e.g., NH2-MIL-125), they can combine with distinct semiconductor substances, such as GQDs with enhanced visible light harvesting ability and effectively endorse in photogenerated electrons transmission and recombination, thus enhancing the photocatalytic performance [43, 44]. However, GQD@MOFs Cos have some limitations in the field of photocatalysis [71, 72], and can be managed by using various synthetic approaches. The applications of QD@MOFs Cos in photocatalytic CO_2 reduction to CH_4, photocatalytic water splitting [72], photocatalytic reduction of Cr^{6+}, degradation of organic dyes, and oxidation of nitric oxide have been described in detail.

5.12 Photocatalytic Activity

5.12.1 Photocatalytic Hydrogen Evolution

Confined lead-free PeQDs@MOFs are used for proton reduction due to visible light interaction [25]. The semiconductor material MoS_2 has been considered an auspicious supplement for the noble-metal photocatalyst [72]. But this material has certain limitations, such as the fact that it can easily agglomerate in the solution, showing weak light absorption capability and poor photocatalytic performance. Therefore, MoS_2 is united with MOFs to produce a MoS_2@MOFs Cos because MOFs with ultra-high surface areas, and organized voids afford a favourable place for MoS_2 molecules to load and prevent their aggregation. MoS_2 QDs@graphene@TiO_2 has been designed by a facile one-step solvothermal route with a noteworthy growth in the photocatalytic activities due to the amplified charge separation and absorbance rate under visible-light [72]. MoS_2QDs@graphene@UiO-66-NH_2 is an excellent candidate for the photocatalytic hydrogen evolution reactions (Fig. 8a) due to the virtuous dispersal ability of the resultant composite. Sometimes, the excess QDs

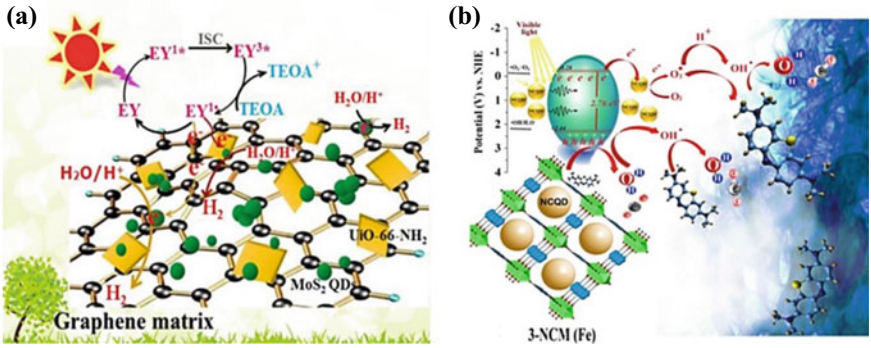

Fig. 8 **a** Possible mechanism for photocatalytic H_2 production, using MoS_2 QDs/UiO-66-NH_2/G under the irradiation of visible light, reproduced with permission from Elsevier [72], **b** proposed mechanism for methylene blue removal, using of 3-NCM(Fe), reproduced with permission from the reference [73]

occupy all the voids of MOFs and even block them, leading to reduced porosity and surface area of QD@MOF. Still, the interactions between QDs and MOFs have maintained enhanced photocatalytic activity [43].

Taking another example, amino acid-based CQDs@MIL-53 (Fe) behave as an extremely effective photoreactor, showing visible-driven photocatalytic activity for methylene blue photodegradation (Fig. 8b). The amino acid-based CQDs heightened the migration of photogenerated electrons and holes. Along with this, holes and radicals of hydroxyl groups are more projecting than the radicals of superoxide throughout the degradation process [73].

5.12.2 Photocatalytic Cr^{6+} Reduction

Major industrial water wastes include typical heavy metal contaminants such as hexavalent chromium (Cr^{6+}) ions [74]. Cr^{6+} is known as an enormously toxic compound to living organisms and causes carcinogenic problems in humans. A major issue with Cr^{6+} is that it is exceedingly soluble in water [75]. Photocatalytic reduction is observed as an efficient strategy to reduce Cr^{6+} contamination to Cr^{3+} by utilizing the semiconductor as a photocatalyst. The resultant Cr^{3+} is environment friendly and critical to the plants and human health [74, 76]. Temporarily, Cr^{3+} can be simply precipitated to form $Cr(OH)_3$ and detached in a neutral or alkaline medium. In recent reports, limited photocatalysts such as ZnO, TiO_2, WO_3 and CdS have been projected to reduce Cr^{6+}. Though these photocatalysts suffer from low catalytic activity due to objectionable factors, such as photocorrosion, severe photogenerated carrier recombination, and UV-limited activation. A facile generation of CQDs@MOF (MOF = MIL-53, MIL-125, UiO-66 (Zr), and MIL-68) behaves as confined electron acceptors for the enhanced Cr^{6+} reduction [76]. Active metal sites in MOFs serve as light absorbers and charge generators, whereas the organic ligands

in MOFs behave as probes to grip the UV light and energy transfer to metal sites. The CQDs behave as the photosensitizers for MOFs under the light irradiation with longer wavelength, and play an important part in improving the photocatalytic performances of CQDs@MIL-53(Fe) [76].

5.12.3 Photocatalytic CO_2 Reduction Reactions

For the CO_2 reduction reaction, proper visible-light responses and energy band structure are useful for potential photocatalysts. In some cases, serious radiative recombination, poor stability, and lesser CO_2 capturing ability have been noted due to the low conversion efficiency. To solve these issues, designing a semiconducting material@MOFs composite as a photocatalyst is required with an improved CO_2 reduction process. In recent reports, a facile *in-situ* procedure has been applied to prepare $CsPbBr_3$@Zn/Co ZIFs for efficient photocatalytic CO_2 reduction [77]. The $CsPbBr_3$@Zn-ZIF or Co-ZIF Cos show enhanced moisture stability, charge separation efficiency, and CO_2 capturing ability. Upon irradiation with visible light, $CsPbBr_3$ QDs shift from ground to the excited state to generate pairs of electron-holes that are easily detached at the catalytic active metal centres in ZIFs, resulting in enhanced CO_2 reduction performance.

In another report, PeQDs@PCN-221 is synthesized by a consecutive deposition method, where PeQDs are captured by the Fe-porphyrin-based PCN-221 and capably used for the photocatalytic reduction of CO_2–CO and CH_4, using water as a sacrificial reductant [78]. The growth of an effective artificial photosynthesis depends on economical photosensitizers and the earth-abundant photocatalysts, to reduce carbon dioxide into feedstocks or fuel. In some reports, CQDs-sensitized semiconductor photocatalytic systems have limitations i.e., low efficiency and high rate of energy consumption due to maximum number of barriers. These limitations are responsible for the kinetically favourable reduction of water rather than carbon dioxide in carbonated water. In earlier reports, significant efforts have been made to hunt for novel CQDs that may overcome the limitations. Rational design and formation of GQDs@Zn-MOFs systems have been done to use them as photocatalysts for effective CO_2 reduction under visible light [79]. Compared with previous GQDs, the GQD@Zn-Bim-His-1 composite exhibits boosted photocatalytic activity for CO_2 conversion (20.9 and 3.7 μ mol h^{-1} g^{-1} are the formation rates for CH_4 and CO, respectively). The synergistic effect between rich active sites in imperfect Zn-Bim-His-1 and GQDs encouraged the photogenerated electron–hole separation in photocatalytic conversion [79].

5.12.4 Photocatalytic Removal of NO

Nitrogen oxides (NO_x) are known as poisonous gases, and nitric oxide and nitrogen dioxide, mainly cause air pollution. These gases create harmful effects on human

health and cause severe environmental damage. Photocatalysis is an auspicious technique and can be used to remove NO at the ppb level. This process can be done with less consumption of energy and operates at normal temperature and pressure conditions [80, 81]. In recent studies, the 2.5 vol% N-doped CQDs@MIL-125(Ti) MOF composite, synthesized via a facile route at room temperature, showed 49% removal efficiency for NO under enhanced visible light [82]. The MIL-125 is showing an energy band gap of 3.6 eV, responds to UV light relative to visible light, thus suffering from fast electron and hole recombination. Consequently, this material cannot be used directly for the effective removal of NO. There is a crucial need to overcome the limitations of MIL-125 [83, 84]. Electrochemical impedance (EIS) information exhibited that the efficiency for charge carrier separation of N-doped CQDs@MOF is suggestively higher than the MIL-125. As additional information, the Ti^{3+}–Ti^{4+} in MIL-125 behaves as an active centre for the photocatalytic NO exclusion. There are two probable pathways for the migration of electrons. First is the transfer of electrons from N-doped CQDs to the Ti^{3+}–Ti^{4+} centre in MIL-125 due to the photoinduced electron transfer property of N doped CQDs. Second is the absorption of UV light originated from N doped CQDs by an organic linker, i.e., terephthalic acid, trailed by the transfer of electrons to active metal sites for the photocatalytic removal of NO.

5.12.5 Enhanced Light Harvesting

In many reports, it has been mentioned that QDs@MOFs Cos can be used as photocatalysts to produce energy and for inhaled light harvesting applications [43, 85]. The designed QDs@MOFs always hold carbonaceous materials to evade the chances of particle agglomeration and barricade the limitations of the light harvesting process. The functionalized porphyrin-based Zr-MOFs with CdSe or ZnS core/shell QDs are the best examples as photocatalysts for enhanced light harvesting by the transfer of energy from QDs to MOFs. In this case, nearly 80% energy has transferred from QDs to MOFs, as measured by time-resolved emission studies. There is a wide absorption band related to the QDs observed in the visible region, which suggests the better coverage of the solar spectrum by CdSe or ZnS@MOF. There is about 50% enhancement observed in the number of harvested photons harvested with QDs coated MOFs [86]. Thus, depending on this Table 1 shows a list of Cos, their type, synthesis techniques and potential applications [87–99].

6 Conclusion and Future Perspectives

This chapter has presented an overview of advancements in these Cos, synthetic approaches, diversified structural features, unique properties, and outstanding applications. MOFs have enormous structural features and chemical stability, which makes them attractive substances for constructing composites with semiconductor-like behaviour containing QDs. Sequentially, QDs have desirable light-harvesting

Table 1 Showing information related to synthetic approaches for QD@MOFs Cos, and their applications

S. No	Application	MOF	QD	QD@MOF composite	Methods of synthesis	Refs.
1	Hydrogen production	MIL-101/ MOF-5	CdS	CdS@MIL-101/MOF-5	Ship-bottle	[52]
2	Hydrogen production	ZAVA	CdS	CdS@ZAVA- MOF	Bottle-ship	[87]
3	Hydrogen production	UiO-66-NH_2	MoS_2	MoS_2 QDs/UiO-66-NH_2/G	Solvothermal	[72]
4	Hydrogen production	ZIF-8	ZnCdS	ZnCdS@ ZIF-8	Template method	[88]
5	CO_2 reduction	PCN-221(Fex)	$CH_3NH_3PbI_3$	$CH_3NH_3PbI_3$@PCN-221(Fex)	Encapsulating technique	[78]
6	Solid state lightning	ZIF-8	CdSe/CdS	CdSe/CdS-PVP@ZIF-8	Solution process	[89]
7	Temperature and aqueous heavy metal detection	MOF-5	$CH_3NH_3PbBr_3$	$CH_3NH_3PbBr_3$@MOF-5	Solvothermal	[90]
8	Chemical sensing	ZIF-8	ZnS:Eu	ZnS:Eu @ZIF-8	One-pot method	[91]
9	Chemical sensing	Zn-MOF-8	Ag	Zn-MOF-8@Ag	Sonochemical technique	[92]
10	Chemical sensing	ZIF-8	GaN	GaN@ZIF-8	Solution process	[93]
11	Chemical sensing (oxidase additives)	ZIF-8	CdTe	CdTe@ZIF-8	Solution process	[94]
12	Supercapacitor	ZIF-8	SnO_2	SnO_2 QDs@ ZIF-8	In-situ epoxide precipitation method	[95]
13	Photocatalytic and photovoltaic	NTU-9	CdTe	CdTe@ NTU-9	In-situ	[96]
14	Photovoltaic	Eu-MOF	CdTe	CdTe QD/Eu-MOF	Solution process	[97]
15	Light harvesting	IRMOF-3	CdSe/CdS/ZnS	CdSe/CdS/ZnS QDs @ IRMOF-3	Solution process	[98]
16	Visual recognition	ZIF-8	CdTe	CDs/QDs@ZIF-8	Solution process	[99]

capacity, optoelectronic, and fluorescent properties. The fashioned Cos have gained approval because of their improved stability, remarkable physio-chemical/opto-electrical features, and broader range of applications such as electrocatalysis, light harvesting, bioimaging, energy storage, etc. MOFs play numerous roles in the stabilization, dispersion of QDs and the modulation of electron-hole recombination by the charge separation. Along with this MOFs also enhance the coordinatively unsaturated metal sites for the accommodation of chemically active species in QDs. MOFs with tunable pores can behave as encapsulating agents to construct well-organized semiconductor particles without any chances of agglomeration. Recently, QDs@MOFs composites have been involved in the areas of energy production, storage, and environment remediation, such as green H_2 production, CO_2 reduction to produce methane, degradation of organic dyes, Cr^{6+} reduction, and NO oxidation etc.

Despite the advantages of QDs@MOFs in various applications, there are still many challenges exist. A major concern in the synthesis of these composites is the control over the morphology and size of semiconductor QDs. It is also vital to stabilize the dispersion ability of the QDs in the solution containing MOF components to get all the profits from both the properties of MOFs and QDs. In recent approaches to fashioning the QD@MOF composites, there has been a random and uncontrolled growth of QDs in the MOF matrix. The controlled synthesis of QDs@MOFs with advanced surface properties is a frightening task. A precise ratio of all precursors, experimental conditions directly affect the size of QDs and placement of organic linkers in MOFs. Then, the progress of QDs@MOFs composite can be accomplished by proper association between the functional groups of MOFs and QDs. Although, major developments have been made in the area of QDs@MOFs-based photocatalysis. There are still a lot of efforts to be made for the industrial-scale production of these type of photocatalysts. Several kinds of QDs have poisonous compositions, especially inorganic QDs contain heavy metal atoms that can cause environmental pollution and human health issues. It is essential to develop low-toxic QDs@MOFs composites from environment friendly precursors such as carbon-based QDs and encapsulate them in MOFs, which have great potential in biomedical applications and lesser environmental risk in other applications. It is also required to encompass the applications of Cos, such as their utilization for removal of organic pollutants such as pesticides and antibiotics as well as detection of heavy metals. Thus, in conclusion, these Cos are a developing class of materials which shows an optimistic future with their great potential for high-impact contributions towards numerous applied research areas.

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Quantum Dots/2D Composites

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Abstract This chapter explores the intriguing domain of quantum dots (QDs) and two-dimensional (2D) materials, considering their synergistic combination to create innovative composites. In this context, the combination or synthesis of 2D materials with low-dimensional nanostructures in intricately engineered frameworks presents a novel opportunity to effectively integrate their distinct features in a complementary way. The combination of 2D and 0D nanomaterials in nanocomposites offers a promising approach to leverage the unique properties of each constituent. Combining the surface property tuning, small molecule attachments, external stimuli sensitivity, robust environmental resistance, negligible interference to other analytes, and recyclability of these materials, the resulting hybrid nanocomposites can overcome the limitations associated with each component synergistically.

Keywords Quantum dots · 2D materials · Nanocomposites · Graphene · MXenes

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1 Introduction

Two-dimensional (2D) nanomaterials are a type of nanomaterial with unique capabilities due to their ultra-thin, planar structure. One of the most well-known examples is graphene, a single layer of carbon atoms organized in a hexagonal lattice. Since the discovery of graphene, there has been a substantial boost in material science research due to the effective exfoliation of single-layer transition metal dichalcogenides. Graphene demonstrates extraordinary electrical conductivity, mechanical strength, and thermal properties [1–3]. Its unique structure and exceptional attributes have paved the way for extensive research and various applications, from electronics to materials science. Besides graphene, there has been a growing interest in exploring other two-dimensional (2D) nanomaterials such as MXenes, including transition metal carbides, nitrides, and carbonitrides, borophene, hexagonal boron nitride (h-BN), and phosphorene [4–12]. These materials possess various electrical, optical, and mechanical properties, rendering them appropriate for multiple applications. 2D nanomaterials are utilised across diverse domains, including electronics wherein they serve as integral components for transistors and sensors, optoelectronics wherein they facilitate the development of photodetectors and light-emitting devices, as well as energy storage wherever they function as electrodes within batteries and supercapacitors [13–16]. The considerable surface area and distinctive electrical characteristics of these materials play a significant role in enhancing their efficacy in catalytic reactions, sensing mechanisms, and biological implementations.

Recent advancements in the synthesis of nanomaterials and the research conducted on nanotechnology have showcased significant implications across all domains of science, engineering, and healthcare. Quantum Dots (QDs) are a significant category of nanoparticles widely utilized in many biological and industrial contexts [17]. The concept of QDs was initially formulated for both solid (glass crystals) and liquid states. The term 'Quantum' denotes a small and distinct unit of a physical attribute. The term of "quantum dots" is derived from the fundamental influence of quantum mechanics on the optical characteristics of nanocrystals [18]. QDs are recognised as a novel class of semiconductor inorganic crystals with dimensions on the nanometer scale. According to the technical definition, they can be described as tiny crystals that contain a variable number of electrons. These electrons occupy distinct quantum states and possess electronic properties that lie between those of bulk materials and discrete fundamental particles. Graphene has several limitations, including limited light absorption, rapid recombination of photo-generated carriers, ease of aggregation, and low dispersion. These shortages make it difficult to use these two materials in future optoelectronic devices [19]. Due to hydrogen bonding or van der Waals interactions, MXenes are prone to self-restacking, resulting in a considerable loss of electroactive area as well as ion and electrolyte inaccessibility [20]. The composites of 2D MXene and QDs offer a promising approach to leverage the unique properties of each constituent. This design enables the tuning of surface properties, attachment of small molecules, sensitivity to external stimuli, resistance to environmental factors, minimal interference with other analytes, and recyclability. Consequently, the

hybrid nanocomposites effectively address the limitations associated with the individual components. The enhanced performance of QDs/2D materials hybrids can be attributed to the induced synergy between the constituent materials. The combination of 2D material and 0D nanomaterials in nanocomposites allows for the effective integration of the unique benefits offered by each component. This includes the ability to modify surface properties, attach small molecules, respond to external stimuli, withstand harsh environmental conditions, avoid interference with other substances, and be easily recycled. By combining these advantages, the hybrid nanocomposites overcome the limitations of each individual constituent in a synergistic manner.

2 Fundamentals of QDs

QDs are nanoscale zero dimensional particles with unique and favourable optical and electrical characteristics due to their quantum confinement phenomena. These tiny structures, typically measuring between 2 and 10 nm in diameter, can be regulated to generate distinct wavelengths of light through size modulation [21–24]. QDs such as graphene quantum dots (GQDs) or carbon quantum dots (CQDs) display advantageous properties, such as high luminescence, broad emission spectra, and resilience against photo bleaching [25–29]. These attributes render them highly beneficial in domains such as medicine for targeted therapy, diagnostics, imaging, energy-efficient screens, and solar cells for increasing light absorption [30, 31]. Furthermore, the ability to functionalize QDs with specific surface coatings allows for their incorporation into a wide range of applications, from biological markers to sensors [32]. While the technology evolves, researchers are looking for new methods to use QDs' unique features to advance materials science, electronics, and medical diagnostics [3, 32–37]. This chapter gives an overview of the latest developments and highlights research results for next-generation hetero structures of QDs/2D Composites.

3 Different Types of Quantum Dots/2D Composites

Graphene, a single sheet of hexagonally organised carbon atoms, offers remarkable electrical, thermal, and mechanical properties [38–42]. A synergetic relationship occurs when QDs, particularly semiconductor QDs such as CdSe or PbS, are integrated. The strong connection between graphene and QDs improves charge transport resulting in photoluminescence and electrical conductivity. Graphene has several limitations, including limited light absorption, rapid recombination of photogenerated carriers, ease of aggregation, and low dispersion. These shortages make using these materials in future optoelectronic devices difficult. Since the synthesis of QDs/Graphene composite materials in 2010, the synergistic effects of the composite materials have overcome the disadvantages of QDs and graphene [43, 44]. Within this hybrid arrangement, the QDs function as light-absorbing substances, generating

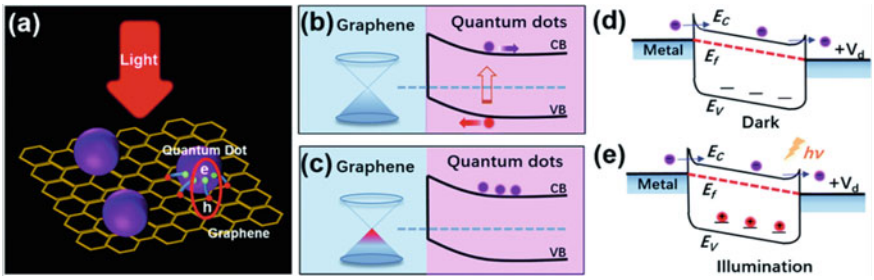

Fig. 1 **a** A schematic representation of the QDs/Graphene composite and light-generated electron–hole pairs. Energy level diagrams of the QDs/Graphene interface demonstrating **b** electron–hole pair production in QDs under illumination and **c** trapped carrier doping in graphene. Diagram illustrating the photogating effect **d** in the dark with external bias and **e** in the light [19]

electron–hole pairs when exposed to light. Subsequently, the carriers can be promptly transferred to graphene, resulting in photocurrent generation. This process facilitates the effective separation of electrons and holes, hence extending the lifespan of the carriers. The photo-generated carriers in QDs can be efficiently transported to the surface of graphene sheets, leading to significant optical amplification, exceptional optical sensitivity, and favourable photo-catalytic capabilities as shown in Fig. 1a–c [19]. The fundamental principle of the QDs/Graphene hybrid system is the charge transfer mechanism. The hybrid exhibits synergetic and cooperative effects, which can lead to the emergence of new phenomena and physical features.

Zhao and colleague utilised GO as a framework to grow oxidized SnO_2 QDs[45]. The functional groups of GO facilitated the oxidation of Sn^{2+} ions, while GO served as a template to enhance selective heterogeneous nucleation. Consequently, SnO_2 nanoparticles were formed and developed on the surface of GO. The SnO_2 QDs integrated with GO composite was synthesized by the hydrothermal method. The TEM image of SnO_2 QDs embedded in GO, exhibiting excellent dispersion and a significant amount of loading. Also, it was discovered that SnO_2 QDs tend to grow in the [001] direction, with (110) surfaces serving as oxygen bridging sites on the SnO_2 (110) plane. Liu et al. synthesised the CdTe QDs–TiO_2–graphene hybrid using a universal hydrothermal technique [46]. The CdTe QDs were synthesised and grown directly on the composite surface of reduced graphene oxide (rGO) and titanium dioxide (TiO_2). The reduced fluorescence in the generated hybrid implies that electrons transfer from CdTe QDs to TiO_2 and graphene.

The ZnO QDs/graphene heterojunction photodetectors are created by printing QDs directly on the channel of a graphene field-effect transistor (GFET). This combination of ZnO QDs' strong quantum confinement and graphene's high charge mobility results in exceptional quantum efficiency (or photoconductive gain) for detecting ultraviolet (UV) light while being blind to visible light. The crucial factor for achieving excellent performance is the presence of a clean van der Waals interface, which enables an effective transfer of electric charge from ZnOQDs to graphene when exposed to UV light. Gong et al. reported a robust ZnO QDs surface activation

process and demonstrated that by systematically engineering the ZnO QDs surface, a transition from zero to extraordinarily high photo responsivity of 9.9×10^8 A/W and a photoconductive gain of 3.6×10^9 in ZnO QDs/GFET hetero junction photodetectors (Fig. 2) can be achieved [47]. The lithium-sulfur battery is highly regarded as a leading candidate for the future generation of energy storage devices, primarily because of its cost-effectiveness and exceptionally high energy density. Nevertheless, the uncontrolled development of Li dendrites and the shuttle effect significantly impede its practical utilization. To address both critical difficulties simultaneously, Yu et al.reported a novel Mo_2C quantum dots (MQDs) attached N-doped graphene functionalized interlayers (MQD@NG). The DFT simulations demonstrate that Mo_2C QDs have a lithiophilic characteristic that allows them to build a fast electrolyte diffusion channel for Li^+ transportation and accomplish uniform Li deposition [48]. Additionally, the polar Mo_2C quantum dots have a high affinity for the chemical adsorption of polysulfides. The lithium symmetric cells with MQD@NG interlayers successfully accomplished dendrite-free lithium deposition over 1600 h, even when subjected to a high current density of 10 mA cm^{-2}. The Li–S cells demonstrate a notable capacity of 1230 mAh g^{-1} at 0.2 C and maintain a remarkably stable cycling performance for over 400 cycles without any noticeable decrease in capacity. This study provided a novel perspective on the development and production of high-capacity safe lithium-metal batteries.

The use of ultratransparent electrodes has garnered significant interest in the fields of optoelectronics and energy technology. Nevertheless, achieving a harmonious equilibrium between energy storage capacity and transparency continues to pose a significant challenge. Yuan and coworkers presented a novel approach using a precisely controlled femtosecond laser to synthesise MXene quantum dots (MQDs) and attach them uniformly to laser reduced graphene oxide (LRGO) [49]. The resulting material exhibits outstanding electrochemical capacitance and extremely

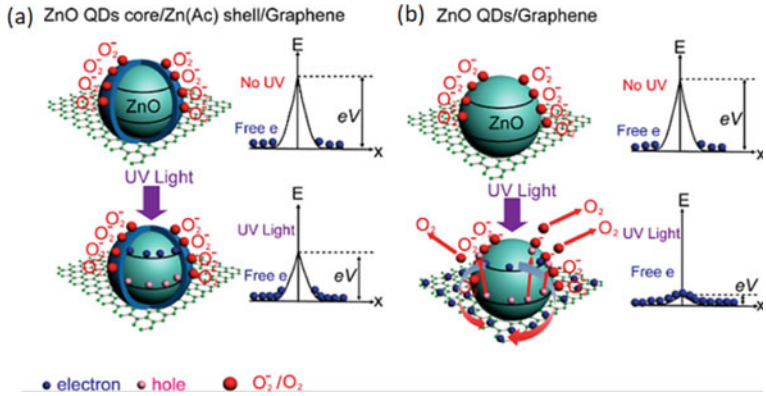

Fig. 2 Schematics of ZnO QDs/GFETs before (**a**) and after (**b**) removing the charge-transfer inhibiting Zn(Ac) shell from a ZnO QD. The influence of the Zn(Ac) shell on the energy band diagram and charge transfer to graphene is also shown [47]

high transparency. An analysis and observation of the synthesis process's mechanism and plasma dynamics occur simultaneously. The inclusion of the unique MQDs in LRGO significantly enhances the electrode's specific surface area by virtue of their nanoscale dimensions and the presence of additional edge states. The MQD/LRGO supercapacitor exhibits exceptional flexibility and durability. It has an ultrahigh energy density of 2.04×10^{-3}mWh cm^{-2}, a long cycle life with 97.6% capacity retention after 12,000 cycles, and an outstanding capacitance of 10.42 mF cm^{-2}. It also offers high transparency, a transmittance above 90%, and excellent overall performance. Moreover, this technique provides a way to fabricate nanostructured composite electrode materials and utilize quantum capacitance phenomena for energy storage.

As two-dimensional substrates for a wide variety of applications, it is anticipated that MXenes, which are both electrically conductive and flexible, would compete with graphene and, in some instances, even outperform itself [50–53]. As a result of hydrogen bonding or van der Waals interactions, MXenes have a tendency to self-restack, which results in a considerable loss of electroactive area as well as inaccessibility to ions and electrolytes [54]. Incorporating spacers, which reside on the surfaces of MXenes, can prevent the MXenes from self-restacking and safeguard their electroactive sites from being sacrificed. This is an intriguing and beneficial concept. In this context, hybridization or composite synthesis of two-dimensional MXenes with low-dimensional nanostructures in carefully designed offers an innovative way to combine their distinctive features in a manner that is complementary to one another [20]. By designing hybrid nanocomposites consisting of two-dimensional MXenes and zero-dimensional QDs, it is possible to effectively combine the benefits of both individual constituents. These benefits include surface property tuning, small molecule attachments, sensitivity to external stimuli, robust environmental resistance, negligible inferences to other analytes, and recyclability. This allows the hybrid nanocomposites to overcome the shortcomings of each individual constituent. Zou et al. described the use of heteroatom-doped carbon dots (CDs) as affordable conductive spacers [55]. The researchers employed a Coulombic self-assembly technique to create free-standing electrodes composed of CDs and MXenes. The fabrication procedure of the CDs/$Ti_3C_2T_x$MXene composite films is depicted in Fig. 3. The CDs have a negative charge, while the MXenes have a positive charge. The resulting electrodes exhibit unique topologies with a combination of 0D (dot) and 2D (sheet) motifs. The selected CDs, varying in particle sizes and heteroatoms, effectively regulated the interlayer spacing and influenced the composition and quantity of the hybrid films. CDs, as intercalators, efficiently reduce the self-restacking of MXene layers and enhance the interlayer distance. This is beneficial for the transport of electrolyte ions and the utilization of the surface active site. A unique design was built employing SnS_2 quantum dots (QDs) and N-doped $Ti_3C_2T_x$ in a 0D/2D heterostructure [56]. The growth process involved solvent spatial confinement and was characterised by its innovative nature. The nucleation and growth of SnS2 nanoparticles were effectively controlled by N-Methyl-2-pyrrolidone (NMP) during the hydrothermal process, leading to the consistent growth of SnS_2 quantum dots with a size of around

3 nm on the MXene matrix. In addition, the generation of NH_3 during the decomposition of the sulphur precursor resulted in the formation of N-doped $Ti_3C_2T_x$, which significantly enhances the transport of Na^+ ions at the interface. The application of this design model leads to the creation of an electrode made of SnS2 QDs and Ti_3C_2, which exhibits exceptional skills for storing sodium. These abilities include a high specific capacity and excellent cycling stability. The synthesis of black phosphorus quantum dots (BPQDs)/Ti_3C_2nanocomposites was achieved by Meng et al. through interfacial assembly [57]. These composites exhibit excellent dispersion of BPQDs that are chemically bonded to MXenes. BPQD/Ti_3C_2 composites are produced by combining BPQDs with $Ti_3C_2T_x$ in NMP by chemical bonding interactions, aided by sonication and stirring. The transmission electron microscopy (TEM) images showed that $Ti_3C_2T_x$ had a morphology consisting of only a few layers, resulting in a high specific surface area. Additionally, the BPQDs were uniformly distributed and had an average diameter of around 4 nm. The BPQDs that are anchored on the Ti_3C_2 nanosheets imparted with increased conductivity and relieved stress upon cycling, which enables a high-capacity and reliable energy storage.

Guo et al. constructed an arrangement of Sb single atoms and QDs on $Ti_3C_2T_x$ aerogels (Fig. 4) for use in potassium-ion batteries (PIBs) [58]. The DFT simulations revealed that the presence of atomically distributed Sb significantly enhances the charge transfer between Sb QDs and MXenes, resulting in improved adsorption performance of K at the interface. Furthermore, the MXene-based aerogel, which possesses a high number of surface functional groups and defects, offers a large number of anchoring sites. This characteristic enhances the structural stability and facilitates efficient electron transport in the composite. Desirable potassium reservoirs require a large loading of Sb, approximately 60.3 wt %, along with short

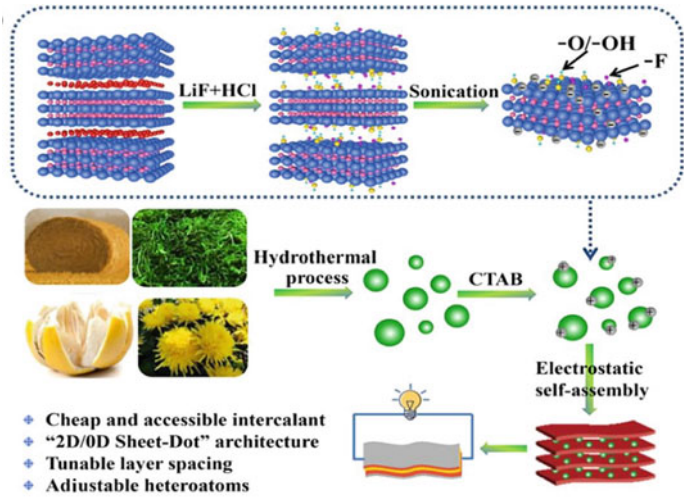

Fig. 3 Schematic of CDs/$Ti_3C_2T_x$MXene composite film fabrication [55]

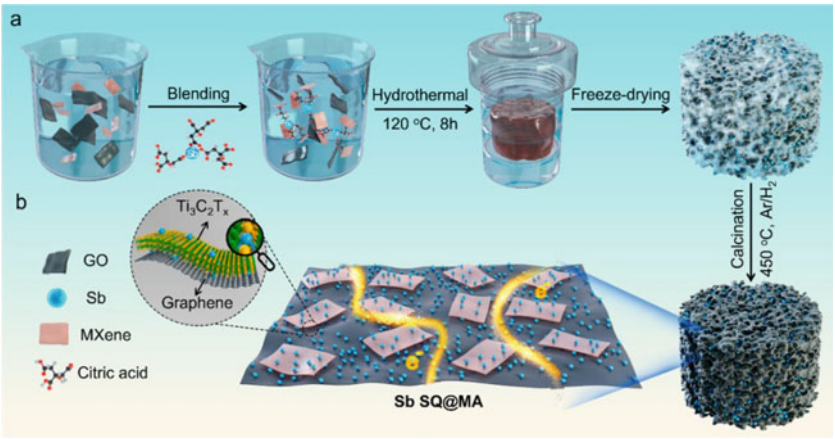

Fig. 4 Pictorial depiction of synthetic methods and structural properties of the Sb SQ@MA composite [58]

ionic transport channels. The combination of these qualities effectively improves the speed and cycling ability of the Sb SQ@MA electrodes in PIBs. This study has presented an illuminating method to customize the interface behavior of hetero structured electrodes for electrochemical purposes.

4 Conclusion

This chapter provides a concise summary of some advancement in the synthesis of multifunctional 2D material and their hybrids with QDs nanoparticles. Chemical components and constructed structures subject to precise manipulation are significant factors in determining the properties of multifunctional QDs/2D nanocomposites. By developing hybrid nanocomposites consisting of QDs/2D nanomaterials, it is possible to efficiently combine the benefits of both individual elements and synergistically overcome the disadvantages of each constituent. Future studies can serve as the foundation for the commercialization of QDs/2D nanocomposites for the purpose of applications in biomedical and environmental applications.

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Quantum Dots/One-Dimensional (1D) Composites

Supriya Rana, Vishwajit M. Gaikwad, and Krishna K. Yadav

Abstract Quantum dots are tiny semiconductor structures that have unique electronic and optical properties due to their small size and quantum confinement effects. In recent years, there has been increasing interest in combining quantum dots with one-dimensional materials, such as nanowires and carbon nanotubes, to create novel composite materials with enhanced properties. These composites have potential applications in a variety of fields, including optoelectronics, sensing, and energy conversion. The integration of quantum dots with one-dimensional (1D) materials can lead to improvements in charge transfer, light absorption, emission, and tunability of electronic and optical properties. The current chapter provides an overview of recent research on quantum dot/1D material composites and their potential applications.

Keywords Quantum dots · Composite · Semiconductor · 1D · Quantum confinement

1 Introduction

The unique properties and unconventional Physics at low dimensions significantly attract scientists' attention in this field. The low-dimensional materials can be categorized into 0D named Quantum dots (QDs), one-dimensional (1D) such as rods, nanotubes and two-dimensional (2D) such as nanosheets [1]. With diameters typically in the nanometer range, QDs exhibit size-dependent characteristics

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such as tunable fluorescence, high photostability, and high quantum yield, making them highly desirable for a wide range of applications in optoelectronics, sensing, and biomedicine [2–4]. By integrating dissimilar materials into a 0D template, heterostructures with varying chemical compositions along the length of 1D materials can be created [5–7]. A typical contact between different dimension metals can be attached in the way shown in Fig. 1 [8, 9]. The interaction between 0D-1D components in these heterostructures results in significant changes in their properties due to variable electronic states, control of charge transport, and the emergence of new physical and chemical properties at their interface not observed in single-component systems [10]. These composites offer several advantages over individual QDs or 1D materials, including improved charge transport, enhanced light absorption and emission, and tunability of electronic and optical properties. Moreover, incorporating QDs into 1D materials can improve their compatibility with biological systems, making them suitable for bioimaging and biosensing applications. In the case of 1D materials, their confined size and high aspect ratio are responsible for their extraordinary properties as compared to their counterpart (2D and 3D). The synthesis of QD/1D material composites involves a combination of bottom-up and top-down approaches, where QDs are synthesized separately and then integrated into the 1D material matrix. Various techniques, such as solution-phase synthesis, vapour-phase deposition, and electrochemical methods, have been developed for the synthesis of these composites. Molecular beam epitaxy (MBE) or metal–organic chemical vapour deposition (MOCVD) growth techniques are also used for synthesising high-quality heterostructure materials; however, this technique requires little mismatch between the two interfaces which should be patterned therefore, only selected heterostructures can be prepared using this technique [11]. In recent years, there have been significant advancements in the synthesis and applications of QD/1D material composites, offering unique properties and promising potential for future research. One of the key advantages of QD/1D material composites is their improved charge transport properties. In individual QDs, electron–hole pairs can become trapped or recombine before they can contribute to charge transport. However, when combined with 1D materials, the 1D material can serve as an efficient pathway for charge transport, facilitating more efficient charge extraction and transport. Additionally, QD/1D material composites can exhibit enhanced light absorption and emission properties. The 1D material can serve as a scaffold for the QDs, increasing surface area and light scattering, which can result in higher photon conversion efficiency in applications such as solar cells and light emitting diodes (LEDs). Moreover, the confinement of charge carriers in both the QDs and 1D materials can lead to improved emission properties, including higher quantum yields and improved colour purity. Another notable advantage of QD/1D material composites is their potential for compatibility with biological systems, making them suitable for bioimaging and biosensing applications. This is particularly important in the field of biomedicine, where materials are needed to interact effectively with biological systems. Additionally, QD/1D composites have been shown to exhibit higher photon conversion efficiencies compared to individual QDs or 1D materials in various types of solar cells. Furthermore, these composites have potential applications in LEDs, where their improved emission properties

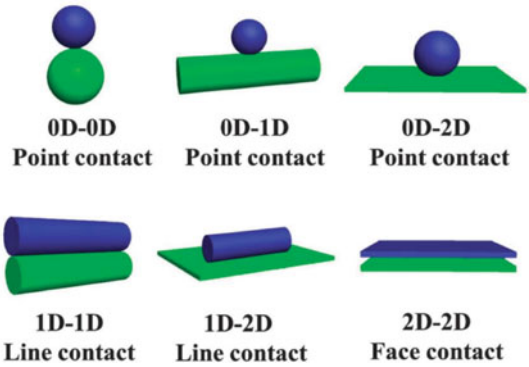

Fig. 1 Schematic diagram of possible combination of MDHs. Reprinted with the permission of ref [8]

can lead to higher efficiency and improved colour purity. Briefly, QD/1D material composites offer unique properties and advantages over individual QDs or 1D materials, including improved charge transport, enhanced light absorption and emission, improved compatibility with biological systems, and potential applications in solar cells, LEDs, and photocatalysis. The synthesis and applications of QD/1D material composites have seen significant advancements in recent years, and their potential for future research and applications is highly promising. 1D materials can be synthesized in different fashions such as single (CNTs) and multi-walled carbon nanotubes (MWCNTs), ultrafine nanorods, nanowires, fibres, pillar-like structures and so on [12–16]. Apart from 1D nanostructures, 0D materials can be also synthesized in mainly two types such as conventional 0D materials and core–shell-like quantum dots [17].

In the current chapter, several modern approaches have been discussed to fabricate quantum dot/1D material composites/heterostructures. Novel QDs/1D heterostructures by incorporating 1D components of titanate nanotubes, metal oxides and metal sulfides, respectively have been overviewed for the first time. The role of 1D structures in tuning local atomic structures to facilitate pathways for effective charge transport is discussed for novel QDs/1D heterostructures. Interface engineering for the manipulation of physical and chemical properties of the composite system is well explained owing to their modern technological applications.

2 Synthesis Route of QD/1D

Various methods have been adopted for the QDs/1D heterostructures synthesis and the approach for synthesis influences the properties of heterostructures. In the following section, we will provide an overview of various synthesis routes of QDs/1D heterostructures. The following sections are classified based on types of different 1D structures and their heterostructures irrespective of quantum dots structures such

as carbon nanotubes with oxide-based QDs or carbon nanotubes with sulfide-based QDs.

2.1 Titanate Nanotubes with QDs

Titanium dioxide or Titania (TiO_2) are highly abundant materials and show their importance in a large range of applications such as catalysis, electronics, water purification and the biomedical field [18]. The properties of titania can be tuned by varying the morphology and can be synthesized in various morphologies such as quantum dots, 1D, and 2D. In the case of 1D morphology, it has been found that protonated titania named titanate nanotubes increase the range of application as well as enhance the existing properties [19]. Titanate can be synthesized via various methods including electrochemical synthesis, microwave irradiation, the hydrothermal method and the sol–gel template approach, where hydrothermal is most popular method due to its large-scale synthesis route as well as economical [20]. Various titanate materials such as protonated titanate nanotubes and sodium titanate nanotubes have been synthesised. Titanates have a hollow structure with accurate inner and outer diameters having a large surface area [18]. Titanates may find application in the fields of catalysis, gas sensing, water purification, energy storage and generation and many others [21]. Titanates are utilized as catalytic support with active species such as metals, oxides, and sulfides.

Recently, Titanate has been used as support for bismuth oxide quantum dots and utilized as the removal of toxic uranium and tetracycline. Sun et al. synthesized the Bi_2O_3 QDs heterostructures with titanates using a hydrothermal route [22]. Pure titanate powder was first synthesized followed by attachment of Bi_2O_3 QDs over titanates using hydrothermal route anchored titanate has been used using hydrothermal route. For the anchoring, different amounts of $Bi(NO_3)_3{\cdot}5H_2O$ were placed into ethylene glycol followed by the addition of ethanol, which is double to ethylene glycol and stirred continuously for 30 min. Afterwards, the desired amount of prepared titanate powder was added to the above solution and stirred for 60 min. The resulting mixture was placed into PolyPhenyLene (PPL)-lined stainless-steel autoclave and heated at 160 °C for 6 h. After completion of the reaction obtained samples were washed and centrifuged with deionized water and absolute ethanol. The end products after being centrifuged were dried in an oven at 60 °C for 12 h. It is interesting to note that this method provides a better attachment of Bi_2O_3 QDs with 1D titanate nanotube. The synthesized heterostructures were characterized using various microscopic and spectroscopic techniques. The transmission electron microscopy (TEM) imaging showed the formation of titanate nanotubes where Bi_2O_3 quantum dots were uniformly distributed over titanate nanotubes. It has been also found that the titanate structures have a very low impact with the addition of different amounts of QDs. The high resolution transmission electron microscopy (HRTEM) study also reveals the attachment of Bi_2O_3 over titanate nanotubes. TEM analysis shows that Bi_2O_3 has an average 2.5 nm diameter, which can be defined as 0D and

these 0D QDs are firmly and uniformly attached to nanotubes and formed 0D/1D heterostructures. The Bi bonded with Ti surface Ti–O–Bi bonds, which will enhance the specific surface area and active site. The removal of uranium was used using an adsorption study and found that it could be 347.39 mg/g. It has been revealed that cation exchange between Na^+ ions and uranium cation is responsible for the adsorption of uranium. The complexation and cation- π interaction of U(VI) is responsible for synergistic adsorption. The synthesized 0D/1D heterostructure efficiently and economically removes the radioactive pollutants and antibiotics from hospital wastewater.

Shi et al. synthesized sodium titanate ($Na_2Ti_3O_7$) nanotube necklaces decorated with bismuth oxyhalide quantum dots (BiOBr) using a hydrothermal route [23]. The BiOBr QDs attachment over $Na_2Ti_3O_7$ necklaces forms a heterogeneous interface between BiOBr (QDs) and $Na_2Ti_3O_7$ (1D). In brief first, 1 g of titania powder was dispersed in aqueous NaOH and heated in Teflon hydrothermal at 120 °C for 24 h followed by washing and centrifugation till 8 pH with water. In the present process, pH plays a very crucial role in each step and the synthesized $Na_2Ti_3O_7$ powder was further dispersed in water and adjusted the pH to 11 by the addition of NH_3H_2O. Thereafter, a certain amount of $Bi(NO_3)_3{\cdot}5H_2O$ and NaBr were added to the above solution mixture and heated at 160 °C for 20 h hydrothermally. The different ratios of BiOBr/$Na_2Ti_3O_7$ composite were obtained after hydrothermal and washed with water and ethanol followed by centrifugation. The scheme for BiOBr/$Na_2Ti_3O_7$ nanocomposites synthesis has been shown in Fig. 2. Different concentrations of BiOBr were deposited over $Na_2Ti_3O_7$. Inductively coupled plasma atomic emission spectroscopy (ICP-AES) studies were performed for the actual loading of BiOBr, which were 4.7, 9.1 and 13.8 wt % and named BOR/NT-0.5, BOR/NT-1.0 and BOR/NT-1.5, respectively. The spectroscopic and microscopic analysis has been performed for the analysis.

The FTIR study reveals the Bi-O bonding (stretching vibration) as well as the stretching vibration of O–Br and O–H bending vibration of surface adsorbed water in BiOBr. In the case of $Na_2Ti_3O_7$, O–H bond and C–H deformation bond were found $Na_2Ti_3O_7$. The Ti–O and Ti–O–Ti vibration bands were also found in $Na_2Ti_3O_7$.

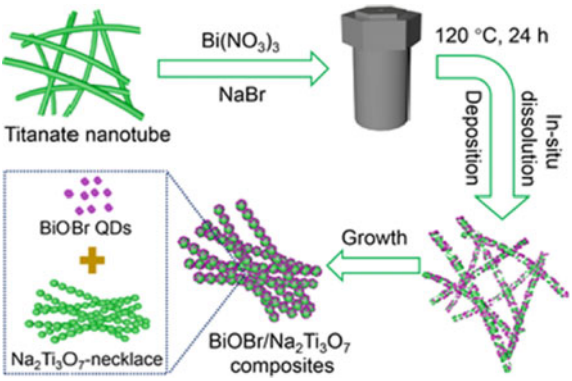

Fig. 2 Schematic representation for BiOBr/$Na_2Ti_3O_7$ necklace composite. Reprinted with the permission of ref [23]

In the case of BiOBr/$Na_2Ti_3O_7$ heterostructures, the existence of few overlapped peaks with somehow shifts unsurprisingly validates the presence of BiOBr/$Na_2Ti_3O_7$ heterostructures. The TEM and HRTEM micrographs show the formation of unique necklace-like morphology interwoven into a network structure. Apart from that it was seen that 2–4 nm QDs of BiOBr are attached to the surface of $Na_2Ti_3O_7$ supports. The synthesized heterogeneous interfaces between BiOBr/$Na_2Ti_3O_7$ decrease electron–hole pair recombination rate and improve the population of oxygen vacancies for capturing electrons. The synthesized BiOBr/$Na_2Ti_3O_7$ interface allows photo-oxidation of benzyl alcohol to benzaldehyde with 100% conversion and > 99% benzaldehyde. The present composite shows substantially higher than previously reported photocatalysts. Apart from these, g-C_3N_4 quantum dots with protonated titanate were developed using a hydrothermal route and utilized for improved photocatalytic hydrogen evolution activity [24, 25]. Out of these results, it has been found that titanates were synthesized using the hydrothermal route and till now the decoration of titanate with various types of QDs was prepared using the hydrothermal route and utilized for catalysis work.

2.2 Metal Oxide 1D Heterostructures with QD

QDs are sometimes referred to as artificial atoms and show improved intrinsic properties as compared to their bulk materials. For instance, when the size of bulk materials is confined to QDs size the bandgap of materials increases due to the widening of the position of the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO), which leads to size-dependent fluorescent materials and absorption properties [26, 27]. Apart from that various other applications were also explored for this type of heterostructure such as solar energy conversion, and catalysis [28, 29]. Chade et al. reported the synthesis of Bi_2O_3 quantum dots (QDs) over $BiVO_4$ nanofibers by direct heat treatment of as-spun fibres. The nanofibers of $BiVO_4$ have 400–700 nm in length where 5–15 nm QDs of Bi_2O_3 were decorated uniformly. The Bi_2O_3/$BiVO_4$ heterostructure shows higher photocatalytic performance than pure $BiVO_4$ nanofibers, which might be due to the enhancement in charge recombination. Lots of work has been done for oxide-based heterostructure synthesis. In another work, Lan et al. synthesized Fe_3O_4 nanowires decorated by CdTe quantum dots [30]. The bifunctional CdTe/Fe_3O_4 heterostructures show enhanced magnetic resonance imaging.

2.3 Carbon-Based Nanotubes/Nanocage Anchored with QDs

Apart from the 1D titanates, other 1D tube-like structures have also been synthesized as a support for various oxides and sulfide quantum dots. From various studies, carbon nanotubes (CNTs) are used as 1D supports. Liang et al. synthesized CNTs with CdS

QDs using a hydrothermal route followed by calcination [31]. Typically, aqueous melamine pH was altered to make it unity by adding phosphorous acid and followed by transferring the mixture into a Teflon-lined stainless autoclave and heated at 180 °C for 10 h and as prepared off-white precipitates after centrifuge used as precursors and calcined in a nitrogen atmosphere for 4 h and denoted as phosphorus-doped carbon nitride tubes (P-CNT). For the synthesis of phosphors-free CNT, the sample obtained after hydrothermal was used at least 30 times with water till the complete removal of phosphors and calcined in N_2 atmosphere in an earlier way. For the synthesis of CdS QDs oil batch method was utilized. $Cd(CH_3COO)_2.2H_2O$ dispersion was made in ethanol using sonication and dropwise addition of thioacetamide (TAA) was carried out during the heating of the oil bath followed by vigorous stirring. The final mixture was centrifuged and washed with deionized water and ethanol several times and dried for further use. The anchoring of CdS QDs over CNTs was done using the in-situ oil bath method. During the synthesis of CdS QDs, a specific amount of synthesized CNTs were inserted after the sonication and followed the previous steps. The morphological study reveals the formation of the well-defined hexagonal tube with smooth and hollow morphology obtained in the case of CNTs, whereas CNTs play a crucial role in anchoring CdS quantum dots over 1D tubes. The energy dispersive spectroscopy shows the presence of C, N, P, Cd and S elements, indicating the successful formation of CdS/P-CNT. The synthesized 0D/1D structures are utilized for photocatalytic H_2 production. It has been seen that there is an almost negligible amount was generated with CNTs, whereas when P-atom incorporation provides a small amount of H_2, it was ~4.5 times higher than P-CNTs in the case of CdS QDs. It is interesting to note that CdS QDs/P-CNT boosted hydrogen production due to their heterojunction, which was responsible for the proton reduction reaction. The production of hydrogen was greatly affected by the loading of CdS QDs. With optimized CdS QDs/P-CNT, a 31.6 times higher amount of hydrogen can be obtained, which is due to the very slow recombination rate of generated electron–hole pairs. However, further incorporation of CdS QDs results in lower hydrogen production due to the shielding effect. Due to the very strong electronic interaction between P-CNT and CdS QDs are the responsible for continuous production of hydrogen with the same amount of up to 15 h reaction under the light.

In another work by Yuan et al. in 2022 synthesized caged-like 1D carbon structures followed by their decoration with vanadium nitride quantum dots and utilized them for sodium-ion capacitors [32]. It has been seen that electrochemical energy storage emerges as one of the significant sustainable energy sources. Lithium-ion and carbon-based materials show their importance in the energy storage sector [33–35]. However, nowadays, sodium ion hybrid capacitors (SIHCs) show their importance in this field due to their high energy density and high output power but the kinetics between anode and cathode limit their performance. Despite being a valid approach, overcoming the obstacle of improving kinetics compatibility between anode and cathode through rational construction of anode materials with pseudocapacitive advantages for fast charge storage has proven to be a significant challenge until now. Concerning the above facts, Yuan et al. developed an advanced solid-state intercalation hybrid capacitors device by coupling freestanding vanadium nitride quantum dots embedded in

nitrogen and fluorine co-doped 1D carbon nanofibers as the anode and corresponding carbon nanofibers as the cathode [32]. For the synthesis of QDs of vanadium nitride over CNTs, the first PVA dispersed was made in hot water followed by the addition of 2 mmol NH_4VO_3 and 3 mL of 60 wt % PTFE solution in PVA dispersion and stirred for another 10 h. The as-prepared dispersion was placed in an ink panel for electro span which was attached to a nozzle connecting a high-voltage power supply and aluminium foil was rolled up on the drum collector to collect nanofibers. The flow rate applied voltage and distance between collector to nozzle was optimized to get good electro-span fibre. The obtained nanofibers film was heated for 6 h at 250 °C then nitridation was performed in NH_3 atmosphere at 700 °C for 3 h and yielded the vanadium nitride QDs heterostructures with CNTs. The utilization of quantum dots confined within 1D nanocages has proven to be effective in overcoming the limitations of sluggish mass and electron transport, resulting in improved kinetics for the anode. Specifically, carbon nanocages co-doped with nitrogen and fluorine, which possess a highly conductive network and abundant active sites, demonstrate remarkable performance in terms of reversible sodium ion storage and anion adsorption/desorption. Consequently, the hybrid device exhibits superior electrochemical properties, including enhanced energy density, power density, and long-term cyclability. This study elucidates a promising strategy to achieve optimal kinetics and performance in energy storage systems. Few other works have been studied where metal oxides, perovskites and graphene quantum dots were decorated over CNTs and utilized for energy and sensor applications [36–39].

2.4 Metal Sulfide 1D Structured with Metal QDs

The synthesis of semiconductor-based materials plays a very crucial role in various applications such as electronics, energy, catalysis, and photocatalysis [40–42]. In semiconductors cadmium sulfide (CdS, $E_g = 2.4$ eV) emerges as an excellent choice for visible photocatalysis and their catalysis properties can be altered by varying the shape of CdS. Earlier, a few reports showed that 1D CdS nanorods will be an excellent choice over another shape for photocatalysis such as charge separation along the axial direction are very well whereas a high aspect ratio can significantly improve the light scattering provided along [43, 44]. As they have several important properties still there will be a scope for improving the performance of the 1D CdS by making heterostructures with metal quantum dots and the high surface area of CdS will provide a better environment for fabrication. By considering this, Chava et al. first time reported the growth of Bi quantum dots (Bi QDs) over the surface of CdS nanorods and utilized them for the photocatalytic degradation of the tetracycline [45]. Briefly, CdS nanorods were synthesized using the solvothermal approach in ethylenediamine at 180°C and for anchoring the Bi quantum dots over it, the synthesized CdS nanorods were sonicated in ethylene glycol followed by the addition of bismuth nitrate pentahydrate precursors and again the solvothermal process was carried out for 2 h at 160 °C. After the reaction samples (CdS/Bi) were obtained

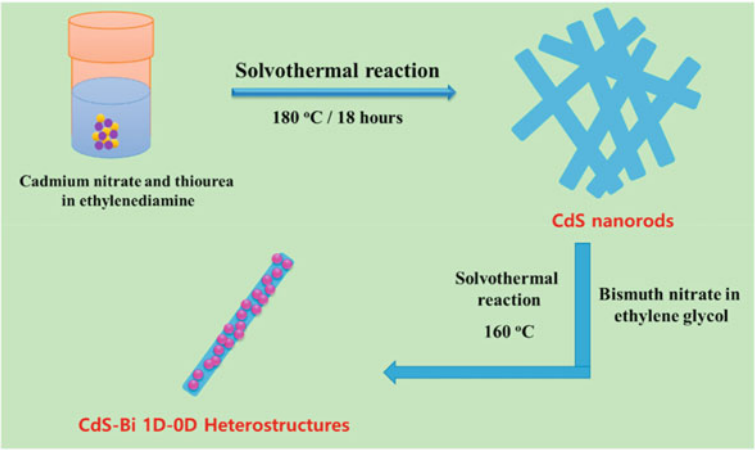

Fig. 3 Synthesis and schematic of Bi nanodots over CdS nanorods. Reprinted with the permission of ref [45]

by washing with ethanol followed by drying. Based on the amount of Bi-precursors used the obtained samples were named CBi-2, CBi-3 and CBi-5. A typical synthesis approach has been reported in Fig. 3.

The main confirmation for heterostructure analysis was performed using the (high-resolution transmission electron microscopy) HRTEM study. From Fig. 4 one can see the HR-TEM images, lattice fringes and SAED patterns of CBi-4 sample (highest amount of Bi). Figure 4a reveals the rods of CdS whereas the rough surface was due to the growth of Bi QDs. From the STEM-EDS elemental mapping, one can see that the Bi-quantum dots were uniformly distributed over the CdS nanorods and confirm the CdS/Bi.

The as-fabricated (CBi-4) heterostructures show the highest photocatalytic activity of tetracycline degradation (90%), which might be due to the synergistic effect of CdS/Bi heterojunction interfaces and surface plasmon resonance (SPR) effect. The fabrication of CdS/Bi plasmonic heterostructures improves the generation of photoexcited charge carriers and decreases the recombination rate of charge.

In another work, Jha et al. synthesized 0D/1D Cu_2O quantum dots sensitized CdS nanorods heterojunction for visible light H_2 generation [46]. For the synthesis of CdS nanorods, Cd and S precursors were inserted into ethylenediamine followed by heating at 180 °C for 48 h solvothermal and yellow precipitate of CdS NRs after reaction can collect via washing repeatedly with deionized (DI) water and ethanol followed by drying at 60 °C for 12 h. For the growth of Cu_2O QD over CdS nanorods, 0.05 M Cu_2SO_4 was dissolved into 10 ml of DI water followed by addition into the above CdS solvothermal precursors and similar steps were used. Different molarity of Cu_2SO_4 was used for making wt % of Cu_2O over CdS and named as x–Cu_2O/CdS (x is the wt % of Cu_2O). A schematic synthesis representation has been shown in Fig. 5.

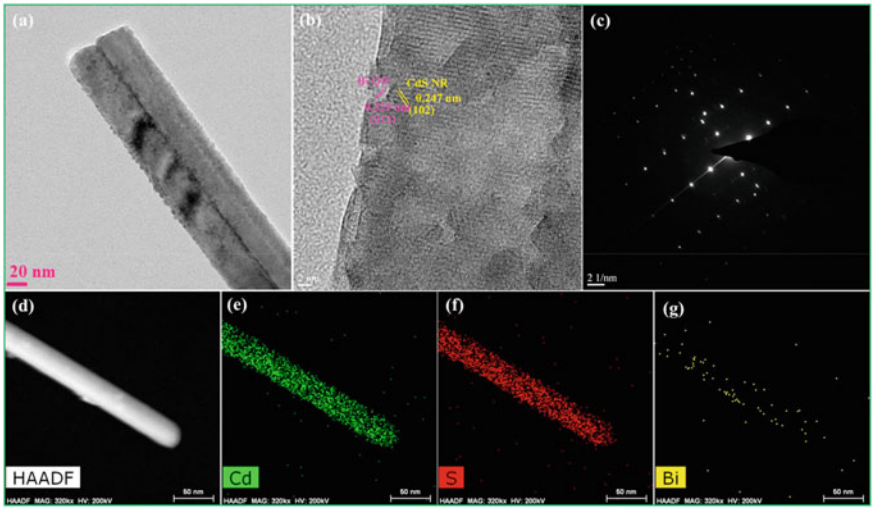

Fig. 4 **a** High-resolution TEM image, **b** inter-planar spacings, **c** SAED patterns and **d–g** STEM-EDS mapping profiles of CBi-4 photocatalyst sample. Reprinted with the permission of ref [45]

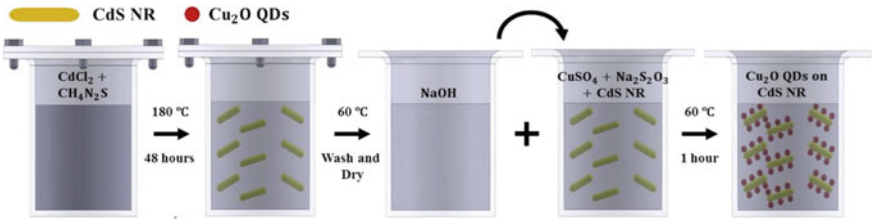

Fig. 5 A schematic diagram describing the synthesis of CdS NRs and Cu_2O QDs/CdS NRs. Reprinted with the permission of ref [46]

The TEM and HRTEM study were utilized for the analysis of Cu_2O/CdS heterostructure (Fig. 6). TEM of 20-Cu_2O–CdS study reveals the formation of nanorods of CdS with Cu_2O QD of size ~4–5 nm (Fig. 6). Figure 6c shows the HR-TEM image demonstrating the incorporation of Cu_2O QDs over the CdS NRs. The lattice fringes also confirmed the formation of Cu_2O/CdS heterostructure (Fig. 6d–e). Figure 6f displays the SAED pattern of Cu_2O QDs/CdS NRs, which confirms the polycrystalline nature of the heterostructure, and the interface between Cu_2O QDs and CdS NRs is abrupt.

The photocatalytic hydrogen evolution (PCHE) test was carried out in the presence of visible light and 20 wt% loaded Cu_2O/CdS shows a high rate of H_2 evolution (130.3 μmol/h, 18 times higher than pure Cu_2O QDs and 5.1 times higher than CdS NRs). The improved H_2 generation over heterostructure arises due to the increased lifetime of photogenerated electrons to 1.2×10^{-8} s.

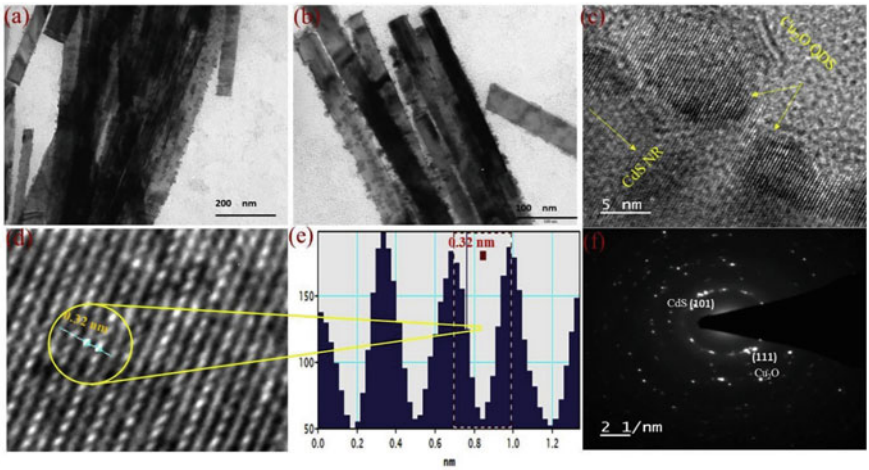

Fig. 6 The TEM image of 20 wt% Cu_2O QDs deposited on CdS NRs (**a**–**c**), Inverse FFT of Cu_2O QDs (**d**), line profile spectra of Cu_2O QDs (**e**), SAED pattern of 20 Cu_2O QDs/CdS NRs (**f**). Reprinted with the permission of ref [46]

3 Future Perspectives

The role of interfaces between the QD/1D heterostructures is yet to be defined. Moreover, there are no clear theoretical models that can guess the behaviour of these systems. Density functional theory (DFT) calculations predict energy levels of QDs shift with different chemical ligands and the binding modes of linker molecules can affect QD coverage and aggregation which have a further impact on charge separation, recombination, and transport properties. Thus, the study of the effect of local nature on these interfaces is important to control the performance of practically possible QD/1D composite systems. The structural and electronic nature of the interfaces would be needed for QD/1D heterostructures with high spatial and temporal resolution. In future, charge transfer at and across composite interfaces in real-time should be needed. A possible way of finding the reason for simultaneously enhanced absorption with optimized light harvesting efficiencies is a big deal. A critical issue remains to build up protocols for reproducibly generating 'pure', homogeneous, crystalline and uniformly well-characterized heterostructures which would be useful for practical commercial device applications. The potential toxicity of QD/1D heterostructures concerns the possible cytotoxicity. It motivated the search for green and sustainable alternatives for the reliable production of individual nanoscale constituent components and heterostructures. In summary, an important future step would be taken towards the spatial integration, stabilization, and ultimately, optimization of these QD/1D composite materials within more sophisticated, realistic device scenarios.

4 Conclusions

The unique properties of QD/1D heterostructures are highly promising as compared to their quantum dots (QDs) or one-dimensional (1D) materials. QDs, which are zero-dimensional materials with nanometer-sized diameters, exhibit size-dependent characteristics such as tunable fluorescence, high photostability, and high quantum yield, making them highly desirable for various applications in optoelectronics, sensing, and biomedicine. By integrating dissimilar materials into a 1D template, heterostructures with varying chemical compositions along the length of 1D materials can be created. The interaction between 0D–1D components in these heterostructures results in significant changes in their properties, including variable electronic states, control of charge transport, and the emergence of new physical and chemical properties at their interface. The synthesis of QD/1D material composites involves combining QDs, which are synthesized separately, with the 1D material matrix. Various techniques such as solution-phase synthesis, vapour-phase deposition, electrochemical methods, molecular beam epitaxy (MBE), or metal–organic chemical vapour deposition (MOCVD) have been developed for the synthesis of these composites. These techniques offer control over the composition and arrangement of the heterostructures, allowing for the customization of their properties. One of the key advantages of QD/1D material composites is tuning the charge transport properties. The 1D material serves as an efficient pathway for charge transport, facilitating more efficient charge extraction and transport compared to individual QDs. The 1D material acts as a scaffold for the QDs, increasing surface area and light scattering, thereby enhancing the photon conversion efficiency in applications like solar cells and LEDs. The confinement of charge carriers in both the QDs and 1D materials can also lead to improved emission properties, including higher quantum yields and improved colour purity.

Acknowledgements KKY thanks SCE for the fellowship.

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Quantum Dots/Bioconjugates

Amandeep Singh, Vandana Dhiman, Kamlesh Kumari, and Patit Paban Kundu

Abstract The quantum dots (QDs) are excellent nanocrystals of prodigious attention to the domain of medical diagnosis and therapeutics. Further, the bioconjugation of QDs shows various unique and worthful optical characteristics which highlight the importance of QDs nanomaterials to be used for very prognosticating fluorescent probes. In the past two decades, super-resolution imaging techniques with different principles have been invented to visualize biomolecules at nanometer scales. This chapter focused on the latest development in the research related to quantum dot bioconjugates fluorescent probe for the super-resolution imaging applications.

Keywords Fluorescent-probes · Bio-imaging · Semi-conductor · Cancer-treatment

1 Introduction

The fluorescent microscopy technology is extensively used in the biotic research. But, the optical diffraction limit restricts the spatial resolution of fluorescent microscopy. Since several years, super-resolution imaging methods with diverse mechanisms are being investigated to envision the biomolecules of nanometer measures. Majorly, the advancement of such techniques is based on the functionality and efficiency of the fluorescent probes. Specifically, the inherent characteristics of the fluorescent probe limit to the optimum imaging performance of the high-resolution

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fluorescent nanoscopy. The recent progress in fluorescent probe bioconjugates for super-resolution imaging techniques includes the bioconjugates based on fluorescent proteins (FPs), organic dyes, quantum dots (QDs), carbon dots (CDs), up-conversion nanoparticles (UCNPs), aggregation induced emission (AIE) nanoparticles, and polymer dots (PDs) [1–9].

The hurdle of chemo/antibiotic resistance in the modern medicine domain remains pertinent and is yet to be overcome. Sensitivity of microorganisms regulates the choice of drugs to be used that eventually influenced the efficiency of disease management as well as prognosis for patient. Though, considering the adaptation procedure of discrete strains of microorganism, unrestrained applications of antibiotics will certainly lead to the maintenance of crisis of antibiotic resistance globally including the realization of a circle which declines the functional and anatomical results of treatment of inflammatory diseases [10].

This chapter describes the physicochemical properties of quantum dots as well as biological nanoconjugates as an option to be used as fluorescent probes cancer treatment. In this chapter, the main emphasis is on quantum dots based bioconjugates fluorescent probes for the high- or super-resolution florescent micro- or nanoscopy. Also, based on the properties of already existing probes and their compliance with present imaging techniques, an outlook for further improvement in fluorescent probes domain for super-resolution imaging is also provided.

2 QDs for Biomedical

The fluorescence emission is the widely explored characteristic of QDs by doping of transition elements in the core of nanocrystals as an approach to be used to impart novel value-added multimodal features to the QDs for biomedical uses. One of the utmost fascinating features of the quantum dots is the uniqueness of their emission spectra that can be delicately regulated, just by changing the shape, size, and material composition of the QDs [11]. Due to the recent progress in bioconjugation chemistry as well as accessibility of various existing constituents for the functionalization, interest is being increasing to combine quantum dots with several other components to develop multimodal composites materials.

Different types of quantum dots include colloidal quantum dots which are used for biosensing, magnetic quantum dots which enable magnetic cell separation, and fluorescent quantum dots which can be utilized for magnetic resonance imaging (MRI).

2.1 Semiconductor QDs

Semiconductor quantum dots emerge as an outstanding material in the arena of biomedical study. Morphologically, the QDs are spherical-shaped semiconductor

nanocrystals having photoluminescent nature and own particle dimensions ranges between 2 and 10 nm. The size of a quantum dot is greater than that of characteristic atomic radius, however, adequately delivers electrons' and holes' quantum confinement in the all of three spatial magnitudes. Subsequently, semiconductor quantum dots, sometimes, are also called artificial atoms. The exceptional characteristics of semiconductor quantum dots include the quantum confined states' energies as well as wave functions those are prone to be altering by changing the size, shape, and composition of QDs.

2.2 Metal-Doped QDs

Metal-based nanoparticles are in the focus of attention due to their massive prospective in the nanotechnology [12–14]. The metal-doped quantum dots can be prepared using hydrothermal, solvothermal, microwave, reduction, and sonochemical techniques. These QDs were found to be excellent antifungal, antibacterial, bioimaging agents, and antioxidant, in nature. Also, these nanomaterials exhibit good photodynamic as well as photothermal therapy properties. Doped QDs not only potentially retain almost all of the above advantages, but also avoid the self-quenching problem due to their substantial ensemble Stokes shift. Various compounds, i.e., ZnS, ZnO, ZnSe, CdS, CdSe QDs etc. are employed as host lattices inside which some additional transition-metal ions or lanthanide ions, i.e., Mn^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+}, Ag^{+}, Pb^{2+}, Cr^{3+}, Eu^{3+}, Tb^{3+}, Sm^{3+}, Er^{3+} etc. are integrated as dopant moieties which turns to upsurge the luminescent properties of metal-doped quantum dotes with advanced features [15].

2.3 Carbon-Based QDs (C-dots)

Carbon-based quantum dots are new generation carbon-based nanomaterial. Recently, the C-dots have fascinated wide research attention due to their miscellaneous physicochemical features as well as promising characteristics, i.e., good biocompatibility, exclusive optical nature, economic, eco friendliness, presence of plentiful functional groups ($-NH_2$, $-OH$, $-COOH$), electron mobility, and significant stability.

3 QD Bioconjugation

Methods for bioconjugation of quantum dots include covalent binding, non-covalent binding, or direct coupling of ligands to coat the surface of QDs. Quantum dots can be attached to DNA, Proteins, and numerous other biomolecules.

3.1 QDs-Proteins Bioconjugation

QD-protein bioconjugates are considered to be influential assemblies for the biosensing and bioimaging as fluorescent probes despite of strenuous procedure for their preparation. The fabrication of QD-proteins bioconjugation assembly takes lots of time and efforts because it involves many steps, i.e., synthesis of colloidal QDs, their solubilization, and thereafter functionalization of QD-proteins bioconjugates.

The attachment of the QDs to the various proteins is determined by the presence of functional groups existing on both the moieties, i.e., QD and respective protein. For instances, the presence of thiols functionalities onto the surface of QD, suggests the bioconjugation with maleimides present onto the protein molecules. Like-wise, carboxyls or N-hydroxysuccinimide (NHS) functionalities present onto surface of QD suggests the bioconjugation with lysine. However, the rarer amino acid, i.e., arginine, is considered for the site-specific bioconjugation. Widely used approaches for the bioconjugation of QDs with proteins are metal-affinity interaction, covalent coupling, as well as avidin–biotin interaction [16].

3.2 QDs-DNA Bioconjugation

The distinctive physicochemical and functional features of DNA have tendered their role as main building blocks. The QDs-DNA bioconjugates are prepared generally by fastening DNA's functional groups with QDs. In general, QDs-DNA bioconjugates are prepared using below-mentioned four different methods.

- Using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) coupling chemistry to attach carboxyl or amine-functionalized DNA to the appropriate groups existing onto surface of QD. The bioconjugation between amine-containing biomolecules and NHS ester brings up the formation of a steady amide bond. In order to react amine with an NHS ester, both are mixed in a polar aprotic solvent, i.e., Dimethyl sulfoxide (DMSO), maintaining a pH level where the amine can get deprotonated.
- A direct attachment of thiolated DNA onto surface of quantum dots through dative thiol interactions. In general, thiol-based bioconjugation process involves the interactions with para-fluoro compounds, maleimides, alkenes, alkynes, halogenated isotactic polypropylene, and vinyl sulfones. Thiol-based bioconjugates are used for various biomedical applications such as to develop biomaterial for drug delivery, to create glycopolymers, and to create various biologically significant monomers, i.e., s-glucuronides.
- Polyhistidine peptide-based DNA assembly to the surface of quantum dots through metal-affinity coordination mechanisms.
- Biotinylated DNA linked to the streptavidin-coated quantum dots.

3.3 QD-Fluorophores Bioconjugation

QDs are widely being used for the energy 'donor' for Forster resonance energy transfer (FRET) pairs. Quantum dots act as FRET acceptors if they are coupled with various polymers capable to act as energy donors, i.e., Polyvinylcarbazole (PVK), Polyaniline (PANI), arylamine-based polyazomethine (TPAPAM), etc. [17]. The conjugates bonded with covalent interactions with the lanthanide derivatives show a greater Stoke's shift, extended fluorescence decay duration, and sharper emission. A huge range of luminescent and quenching compounds are available for FRET applications. Apart from the intrinsic fluorophores of different proteins, i.e., tryptophan and tyrosine; general organic fluorescence dyes, i.e., pyrene, coumarin (UV emission), fluorescein, rhodamine, cyanine (Vis to NIR emission) are extensively used as FRET acceptors as well as donors [18].

4 Applications as Fluorescent Probes

Cancer is fast raising fatal ailments that distress about 18.1 million individuals globally in 2018. Numerous conventional methods, i.e., surgery, radiation, chemotherapy, etc. are used as a conventional cure management for the patients. But, use of these methods bars several restrictions, i.e., development of multi-drug resistance by tumor cells, cytotoxicity (due to off-targeted action), poor intra-tumor localization, physical and psychological stresses, etc. Such restrictions have inspired the researchers to put the effort to develop patient-oriented precision treatment expending progressive drug delivery methods, i.e., liposomes, NPs, nanoconjugates, etc. [19]. Though, such drug transporters too experience various restrictions, i.e., deprived biocompatibility, smaller payload capacity, dripping of the encapsulated drug, and short-range stability. Therefore, the development of biomacromolecule-functionalized nanoconjugates is required to potentiate the anticancer action of healing agents for different cancers, i.e., lung, colorectal, ovarian, breast, and liver cancer. However, the investigators have shown the interest in the biofunctionalized nanoconjugates due to several benefits i.e. good biocompatibility, site specificity with good localization, greater entrapment along with durable stability and also slighter off-target toxicity. The advanced development in the field of biomacromolecule nanoconjugates shall surely inspire the further investigation to develop effective carriers for drug-delivery.

The colloidal quantum dots are considered to be good fluorescent probes having different optical properties far better than organic dyes for the purpose of microscopy imaging. Zhou et al. [20] has used hydrofluoric acid (HF) etching passivation method to develop small nonblinking core-multishell QD. This approach consists of total four phases. First; preparation of InP core, second; preparation of oil-phase core-multishell quantum dots using HF and trioctylphosphine etching passivation method, third; the increment in water solubility by modifying the QDs by 3-mercaptopropionic acid and forth is increasing the capability of bio targeting

using the surface coupling technology. The results revealed that the synthesized non-blinking quantum dots show good fluorescence efficiency, decent photo-stability, significant Stokes shift (greater than 135 nm), and good bio-compatibility along with good targeting. The infectious keratitis causes the weakening of visual functioning which may leads to the monocular blindness with time. By putting the causing factors of eye infectious, inflammatory diseases, antibiotic resistance, and internal mutations of the pathogens together, it has been felt that highly effective alternatives are required to combat the diseases related to eye infectious. For this purpose, fluorescent semiconductor nanocrystals are considered to be potential candidates to treat infectious diseases. Olegovich et al. [21] has evaluated the anti-infectious potentiality of CdTe/Cd MPA 710 QD bioconjugates as well as levofloxacin to counter the *staphylococcal* corneal infection. The results show significant possibilities to consider synthesized QDs to fight against the infectious keratitis diseases.

Furthermore, Rahmani et al. [22] have synthesized QD-DNA conjugates using a single step ligand-exchange approach to overcome the existing problems in preparing stable, bright, and tiny-sized DNA-functionalized QDs. The parameters, i.e., ligand-to-nanoparticle molar ratio, pH, and solvent composition, were varied to synthesize highly photo-luminescent and water-soluble QD-DNA conjugates. The prepared QD-DNA bio-conjugates showed significant colloidal stability against the DNA-directed self-assembly. Furthermore, the fluorescence quenching was done using gold nanoparticles (AuNP) was demonstrated and found that these QD-AuNP dimers can be used as biosensors with excellent sensitivity.

In another study, Savage et al. [23] has developed N−, C− and N−/C-naphthyl substituted hydrophobic amino acid sidechains with Val-and Phe and analyzed them for their propensity for the in vitro self-assembly. The analysis showed the existence of bio-conjugates in both the phases; solution state and solid state. In order to induce the self-assembly, the prepared bio-conjugates are supposed to exposed to the sonication or temperature variations. The bio-conjugates were found to be soft gels of widespread fibrillar linkages of almost same morphology which depends upon the bioconjugate, organic solvent being used, and stimuli provided. Out of six, three formulations of bioconjugates named as 2-NAP-Val-OMe, 2-NAP-Phe-OMe, and Boc-Val-1-NAP were not found to be undergo for the gelation. The forth bio-conjugate (Boc-Phe-1-NAP) has formed opaque soft gels when non-polar solvents were used along with temperature stimuli (without sonication). Furthermore, the di-substituted bioconjugates; 2-NAP-Val-1-NAP and 2-NAP-Phe-1-NAP showed the gelation. Bioconjugate 2-NAP-Val-1-NAP turns to soft transparent gel in benzene solvent on temperature treatment; therefore this bioconjugate can be doped with QDs as well as with highly fluorescent organogel. These results show that the prepared bioconjugates assemble themselves into the ordered structures.

The *Pseudomonas aeruginosa* is considered as one of the dangerous pathogen having fatal characteristics which leads to infectious diseases in eyes. Ponomarev et al. [24] has demonstrated an investigation on anti-infectious activity of bioconjugates based on QDs namely KTCdTe/CdMPA710 and KTInP/ZnSe/ZnS650 in cooperation with the 3rd generation cephalosporin (Cefotaxin) against the hospital-originated *Pseudomonas aeruginosa* strains. The results of the study showed that

the use of KTCdTe/CdMPA710 and KTInP/ZnSe/ZnS650 quantum dot conjugates considerably raises the ZZR.

Furthermore, Manoj et al. [25] have developed an approach to conjugate the InP/GaP/ZnS quantum dots using bovine serum albumin globular protein which create stable QD-BC. The fluorescence polarization analysis shows that the bound-protein holds chromophoric acceptors. A decrease in donor's emission intensity was observed when InP-BSA segment was excited in the presence of bound-chromophores with a simultaneous rise in acceptor's emission.

Dutta et al. [26] have developed an electrochemical immunosensor based on the nitrogen doped graphene QD (N-GQD) as well as single-walled carbon nanohorns (SWCNHs) for α-fetoprotein (AFP) detection which is a cancer biomarker as shown in Fig. 1. Therefore, to construct the immunosensor, nanocomposite material was decorated with N-GQD onto surface of SWCNHs. Subsequent N-GQD@SWCNHs hybrid structure acts as a good base for the immobilization of antibody (Anti-AFP) by carbodiimide reaction having good bioactivity and stability. Immunosensor was fabricated by consistently dispensing the bioconjugates (N-GQD@SWCNHs/Anti-AFP) dispersion onto surface of glassy carbon electrode, and consequently obstructing the residual active sites by bovine serum albumin (BSA) to avert the nonspecific adsorption. In optimum circumstances, the immunosensor showed a wide dynamic range from 0.001 to 200 ng/mL and a little detection limit of 0.25 pg/mL. Moreover, the sensor exhibited greater selectivity, appropriate constancy, and reproducibility. The assessment of AFP in the human serum suggests an exceptional recovery, i.e., 99.2 and 102.1%. Therefore, this study and approach showed a prospective role of nanocomposite-based sensor for the early medical screening of cancer biomarkers.

Salgado et al. [27] have prepared functionalized hybrid semiconductor bioconjugates using fluorescent QD capped by chitosan along with chemically modified with O-phospho-L-serine (OPS) which are biocompatible for human cell sources. Conjugation with OPS leading signaling molecule permits the preferential accumulation in human bone mesenchymal stromal cells (HBMSC). Chitosan shell with fluorescent CdS core was characterized using transmission electron microscope (TEM). Result showed the presence of small sized particles (2.3 nm) as well as a stable photoluminescence emission band. The in vitro biocompatibility results were found to be depended on the surface modification. Also, the efficiency of cells to uptake the NPs depends upon the nature of cells and source of tissue. However, the cellular uptake pathways used during the cell labeling with NPs remains free from the normal cellular biology i.e., adhesion, proliferation, osteogenic differentiation, gene expression, etc. Evaluation of uptake of bone cells exhibits a probable pathway by Caveolin-1 which controls the cell transduction in membrane. Researchers have claimed that the OPS-modified bioconjugated QD evidenced to be a consistent and well-established fluorescent bioprobe for the cell imaging and targeting research that may help to clarifying cellular mechanisms of particles in cytoplasmic membrane and osteogenic differentiation induction. Also, HBMSC's in vitro biocompatibility results showed that OPS-modified chitosan QDs possesses a forthcoming future in laboratory as well as pre-clinical uses, i.e., analysis of bio-images and *ex-vivo* cellular assessment of biomedical implants.

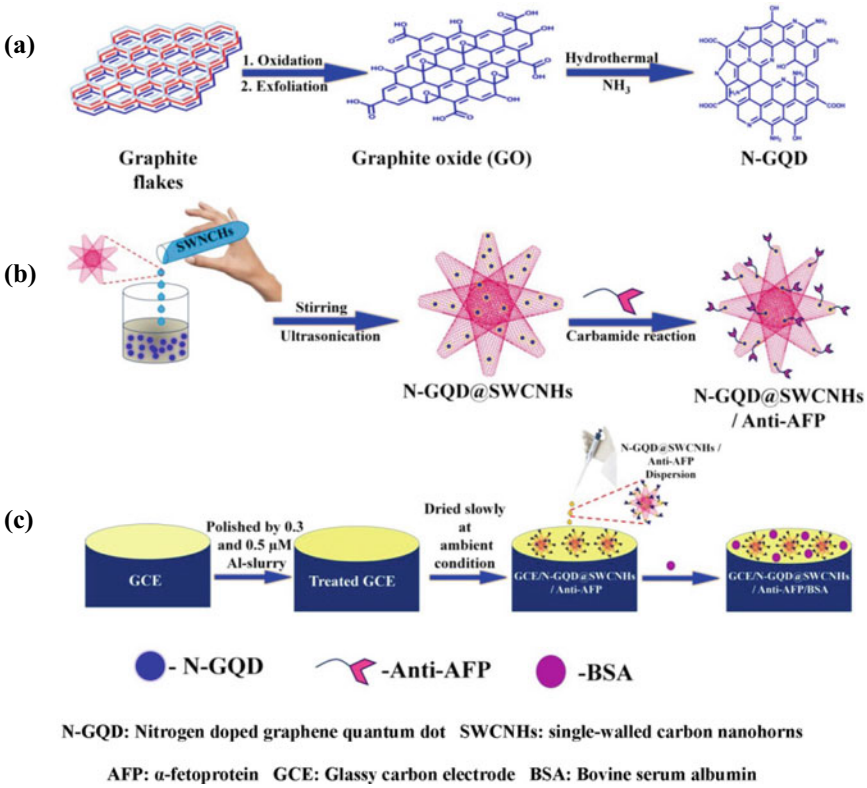

Fig. 1 Preparation of GO and N-GQD from graphite flakes (**a**), preparation of N-GQD@SWCNHs and N-GQD@SWCNHs/Anti-AFP (**b**), and fabrication procedure of immunosensor GCE/N-GQD@SWCNHs/Anti-AFP/BSA (**c**). Reproduced with permission from [26]

The applications of NP bioconjugates to regulate membrane ion channel's activity appeared as a new scope for the cells. Recently, a nanoparticle-based approach is proposed by Nag et al. [28] for the activation of channelrhodopsin C1V1 (ChR-C1V1) expressed in plasma membrane of HEK 293 T/17 cells. The hydrophilic CdSe/ZnS core–shell semiconductor QDs gets self-assembled towards ChR-C1V1 through the metal affinity-driven interaction between QD ZnS shell and N-terminal hexahistidine tag. Research revealed that the laser-induced opening of ChR-C1V1 channel in photoexcited QD activates the ChR-C1V1 channels which was established from a voltage-sensitive dye named bis-(1,3-diethylthiobarbituric acid) trimethine oxonol. Outcomes of the study shows the capability to control interface QDs with living cells for the ultimate activation of ChR membrane proteins.

Biocompatible methods to label the bacteria with fluorescent NPs are indispensible to develop bacterial-bioconjugates for various applications, i.e., biosensors, imaging, medicine, etc. Dong et al. [29] have reported that direct conjugation of carboxyl QDs with outer membrane of *Escherichia coli* through the surface-displayed

binding peptides. Histidine-containing peptide (H6G9) was displayed at N-terminus of membrane-embedded circularly permuted outer membrane protein. Existence of binding peptide makes a milieu different from negatively charged *E. coli* surface and offers resilient binding affinity to carboxyl QDs. TEM results showed that E. coli-QD bioconjugates have higher loading densities immobilized onto cell surface even if loading concentration of QDs is very low (10 μg/mL) to decrease the exposure of cells. Such hybrid cells intensely fluoresce with all different colors of QDs with various emission wavelengths that could be simply imagined using fluorescence microscopy. *Escherichia coli*-QD bioconjugates were found to be highly feasible and preserved the capability to grow and split. In this research, authors claimed that a simple, direct, and highly efficient method can be used to label the bacteria using QDs deprived of considerably negotiating the vitality of cells.

Roy et al. [30] have presented an excited way for the targeted bioimaging of folate receptor (FR)-positive cancer cells by folic acid and MoS2 quantum dots. Water-soluble MoS2 quantum dotes (sized 4–5 nm) along with cysteine functionalization were prepared using a simple bottom-up hydrothermal approach. MoS2 QDs showed the blue emission with highest emission intensity at 444 nm. MoS2 QDs were found to be sensitive for folic acid to yield an active as well as stable nanofiber structure using supramolecular interaction that showed approx. 97% quenching of fluorescence as shown in Fig. 2. High sensitivity and selectivity of MoS2 QDs for folic acid make MoS2 QD-based nanoprobe a suitable applicant for folic acid-targeted imaging probes for in vivo investigation of folic acid-pretreated FR-overexpressed cancer cells. Confocal microscopy images showed that folic acid-pretreated B16F10 cancer cells exhibits greater population of darkened fluorescence as compared to untreated cancer cells as well as HEK-293 cells. Flow cytometry investigation exhibits the substantial variance in mean of fluorescence between the folic acid-pretreated and untreated B16F10 cancer cells. Therefore, such MoS_2 quantum dots-based nanoprobes could be used as potential material for pre-diagnosis of cancer using targeted bioimaging.

Efficient intraoperative identification of cancers involves the growth of bright, minimally-toxic, tumor-specific near-infrared (NIR) probes as contrast agents. Luminescent semiconductor QDs suggest numerous unique benefits for in vivo cellular imaging by giving bright and photostable fluorescent probes. Yakavets et al. [31] have offer the synthesis process of ZnCuInSe/ZnS core/shell QDs emitting in NIR (~750 nm) conjugated with NAVPNLRGDLQVLAQKVART (A20FMDV2) peptide in order to target αvβ6 integrin-rich head and neck squamous cell carcinoma (HNSCC). Result has showed the potential of these synthesized QDs from time-gated fluorescence imaging of stroma-rich 3D spheroids located onto the mm-thick tissue portions to mimic imaging conditions in tissues. Therefore, QD-A20 can be taken as highly capable nanoprobes for imaging-guided surgery as well as NIR bioimaging.

Since optical properties and conjugation capability with biomolecules are two advantageous factors of photoluminescent QDs, thus, are extensively being used in biomedicine domain. Though, the various derivatives of bioconjugation can impacts the interaction between nanoprobes and the targets. Also, the adaptation of size exclusion chromatography to separate the QDs and their derivatives is difficult because

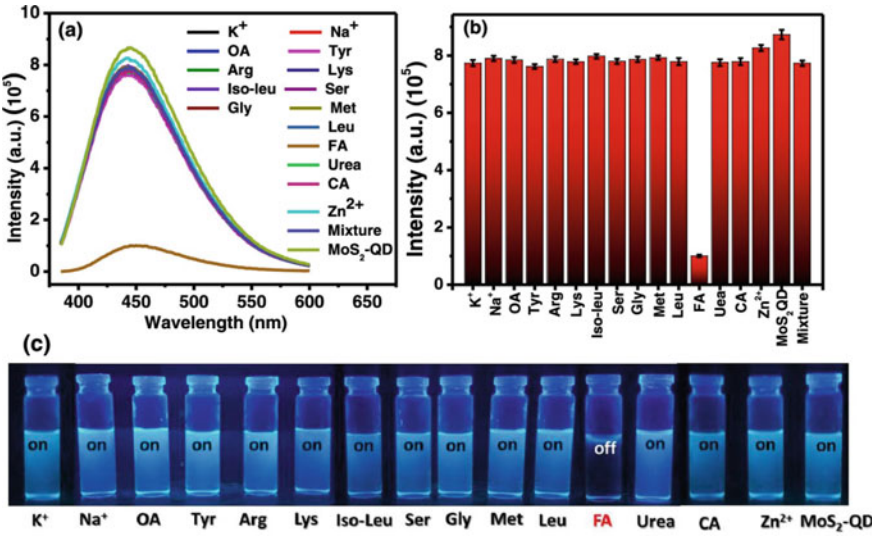

Fig. 2 **a** Emission spectra of MoS2 QDs in the presence of diverse potentially coexisting substances, **b** corresponding bar plot, and **c** photograph of emission images of MoS2 QD solutions in presence of different materials under 365 nm ultraviolet light irradiation. Reproduced with permission from [30]

of the variance in physical as well as chemical properties of biomolecules and NPs. In that regard, Linkov et al. [32] have presented a comprehensive procedure for size exclusion chromatography purification of water-soluble CdSe/ZnS QDs and QD-conjugates by choosing Sephadex resins of various porosities. Efficiency of SEC purification can also be measured by using the PEG derivatives and two types of small molecules named bisnetropsin and 4,5,9-trisubstituted acridine. The results showed that the use of common chromatographic media in group of Sephadex resins permits effective distillation of QD bioconjugates from the contaminants for their successive use for the bioimaging and diagnostics.

MicroRNAs are considered to be imperative post-transcriptional regulators and have great potential to work as noninvasive biomarkers for the disease diagnosis and biomedical research. Although, the multiplex and sensitive detection using microRNAs can expedite the accuracy as well as prompt clinical analysis, but the problem lies that the currently available techniques are generally conceded by taking organic dyes as signal probes, difficult enzymatic and chemical manipulations, and involving complex reaction mechanisms. Therefore, Ma et al. [33] have presented a siRNA-based self-assembled QD biosensor for the facile as well as instantaneous detection of multiple microRNAs. Mechanism wise, the attachment of microRNA targets to the respective QD nanoprobes causes the construction of siRNA duplexes. It further brings the spectrally resolved coding of microRNAs and also enables the association of QDs magnetic NPs bioconjugates in order to isolate and enrich the target microRNAs. However, the disassembled QDs are delicately sensed by the single-molecule detection method by facilitating the sensing of microRNAs at the

level of single-particle. Interestingly, the biosensor recognizes only QDs as signal reporters that instantaneously detect several microRNAs from same specimen and accomplishes femtomolar level sensitivity as well as single-base misalliance selectivity deprived of the participation of the target labeling or/and amplification phases. The same can also be effectively used for the simultaneous detection of microRNAs present in the clinical serum specimen. The finding holds a great evidential supports for the microRNAs turn to be an excellent candidate for non-invasive biomarkers probe for the early disease diagnosis.

Fundamentals to get the most out of the optical properties of colloidal semiconductor QDs in the biological research includes the achievement of effective surface functionalization along with suitable bioconjugation approaches [34]. Since, the functionalization using dextran is greatly effective if used with some NPs materials, but, so far, has shown very restricted uses with QDs. Rees et al. [35] have reported the synthesis, characterization, and applications of dextran-functionalized quantum dots. Various methods for the dextran-based ligands were assessed, including the suitability with the colloidal at various pH values, general attachment with the proteins as well as cells, and the micro-injection to the cells and their viability assays. Different bioconjugation methods were used such as covalent coupling to make a facile pH sensor, attachment of polyhistidine-tagged peptides to quantum dots for the purpose of energy transfer-based proteolytic activity assays, as well as attachment with the tetrameric antibody complexes in order to permit a sandwich immunoassay, cell immunolabeling, and imaging. The outcomes of the study revealed that dextran ligands are extremely encouraging for functionalization of quantum dots and the design of ligands is adjustable to assist the desires of the applications.

Additionally, the circular economy concept's point of view should also be considered whenever a novel material id developed. The overall cost of the raw material as well as processing, their availability, sustainability towards end-use, and recyclability is must to take under consideration.

5 Conclusion

This chapter describes the physicochemical features of quantum dots and biological nanoconjugates for fluorescent probes. The chief highlighting of this chapter is on quantum dots based bioconjugates fluorescent probes for the high- or super-resolution florescent micro- or nanoscopy. Also, based on the properties of already existing probes and their compatibility with present imaging techniques, an outlook for further improvement in fluorescent probes domain for super-resolution imaging is also addressed. The conjugation process fascinates the attention of researchers as it enhances the existing qualities of medicinal products and imparts new properties. Conjugated peptides or proteins, when chemically linked, possess notable characteristics including altered pharmacokinetics, enhanced permeability and safety, and the ability to penetrate challenging areas such as the blood–brain barrier through transcytosis and intracellular delivery. Moreover, chemically-modified peptide/protein

modifications for quantum dots/bioconjugates have a significant impact on the therapeutic domain, owing to their wide range of uses in several domains like oncology, ophthalmology, neurology, immunology, muscle diseases, endocrinology, and more.

6 Future Perspectives

Primary challenge associated with quantum dots/bioconjugates is their intricate nature. However, this problem can be overcome by carefully selecting and optimizing the peptide/protein, linker, and polymer payload. Moreover, it is necessary to carry out computational and theoretical investigations to substantiate the mechanisms of peptide or protein interactions with various polymers in order to investigate their stability and biological activity. Novel experimental and computational techniques will be employed to investigate the structural characteristics of both biological and synthetic constituents of a bioconjugate. This will facilitate a more streamlined understanding of the relationship between structure and function, enabling the fine-tuning of bioconjugates for specific applications.

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Cytotoxicity of Quantum Dots Based Nanocomposites

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Abstract The present chapter provides an overview of the cytotoxic effects of nanocomposites containing quantum dots (QDs) in the context of their biomedical applications. Additionally, this chapter delves into the various approaches that have been proposed to counteract the potential risks associated with the use of QDs. In this discourse, we examine the physicochemical characteristics of QDs, the determinants that contribute to their cytotoxicity, and the surface functionalization approaches utilised to enhance their biocompatibility. The necessity for standardised protocols and regulatory frameworks to guarantee the safe utilisation of the aforementioned is also emphasised. The present chapter provides significant perspectives for

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researchers and clinicians who are engaged in the creation of secure and efficient nanocomposites based on QDs.

Keywords Cytotoxic effects · Quantum dots · Surface functionalization · Biocompatibility

1 Introduction

The scientific community has shown considerable interest in quantum dots (QDs) and nanocomposites as two distinct categories of nanomaterials, owing to their distinctive characteristics and potential utility in diverse domains. Quantum dots (QDs) are semiconductor nanocrystals with dimensions ranging from 2 to 10 nm [1–3]. Their fluorescence properties are determined by their size and composition, which enable them to emit light at different wavelengths. Quantum dots (QDs) have been utilised in various fields such as biological imaging, sensing, and photonics due to their exceptional photostability and adjustable fluorescence characteristics [4]. Nanocomposites are a type of composite material comprising a matrix material and one or more nanoscale components, such as nanoparticles, nanofibers, or nanotubes that are uniformly distributed throughout the matrix material [5, 6]. Nanocomposites have garnered significant attention due to their potential to augment the mechanical, electrical, or optical characteristics of the matrix material at the nanoscale level. This renders them a promising candidate for application in various domains, including aerospace, energy, and biomedical engineering [7]. The amalgamation of quantum dots (QDs) and nanocomposites has resulted in the emergence of innovative nanomaterials that exhibit superior characteristics. These include fluorescent nanocomposites that are suitable for bioimaging, as well as nanocomposite materials that can function as sensors for diverse applications [8].

Nanocomposites based on quantum dots (QDs) have become increasingly important due to their exceptional properties and potential applications [9]. It is important to note that the cytotoxicity of these materials should be considered when using them in biomedical applications. Studies have demonstrated that QDs can cause harm to cells both in laboratory settings and in living organisms [10]. The degree of toxicity is influenced by factors such as the size of the QDs, their surface chemistry, and the concentration at which they are present [11]. The cytotoxicity of QDs can be affected by the matrix material and manufacturing process when they are integrated into nanocomposites [11]. Hence, it is crucial to conduct a comprehensive examination of the cytotoxicity of nanocomposites based on quantum dots prior to their utilisation in biomedical applications [12]. In order to reduce the harmful effects of QDs based nanocomposites, different surface modifications have been investigated [13, 14]. One of the methods is to coat the QDs with biocompatible materials. Lowering the concentrations of QDs in the nanocomposites and optimising the manufacturing process are effective ways to reduce the cytotoxicity of the materials. Although QDs based nanocomposites have the potential to be cytotoxic, their distinct properties

make them appealing for a range of biomedical applications. QDs-based nanocomposites have been extensively studied for their potential applications in targeted drug delivery, bioimaging, and tissue engineering. These materials have the potential to enable controlled drug release, high-resolution imaging, and improved mechanical and optical properties [15–17]. Furthermore, the significance of nanocomposites based on quantum dots in biomedical applications cannot be emphasised enough. It is important to carefully consider the cytotoxicity of these substances to ensure their safe use in vivo [6]. Ongoing research and development suggest that nanocomposites based on QDs have the potential to provide novel solutions to existing biomedical and environmental problems [6, 14, 18–22].

It is crucial to evaluate the cytotoxicity of nanocomposites containing quantum dots (QDs) to ensure their safety and effectiveness in biomedical applications. Quantum dots (QDs) are nanocrystals made of semiconductors that possess distinctive optical and electrical properties. These properties make them highly desirable for a wide range of applications, including bioimaging, drug delivery, tissue engineering, and environmental applications [23–28]. The potential cytotoxicity of these materials is a significant concern that requires attention before their application in biomedical fields. The toxicity of quantum dots (QDs) is influenced by several factors, including their size, surface charge, and concentration [29]. The cytotoxicity of QDs can also be influenced by the matrix material and the manufacturing process when they are incorporated into nanocomposites. It is crucial to evaluate the cytotoxicity of nanocomposites containing QDs by utilising both in vitro and in vivo models before implementing them in human applications. To assess the potential toxicity of nanocomposites, in vitro models involve testing them on cultured cells. These tests can assist in identifying the ideal concentration and duration of exposure for the nanocomposites, which would not result in any harmful impacts on the cells [30]. The evaluation of biocompatibility and safety of nanocomposites is conducted through in vivo models, which entail testing on animal subjects [31]. These tests can assist in identifying the possible harmful impacts of nanocomposites on various organs and tissues. Evaluating the cytotoxicity of nanocomposites made with QDs is crucial to verify their safety for use in biomedical applications. The potential applications of these could include targeted drug delivery, bioimaging, and tissue engineering. The creation of safe and efficient nanocomposites using QDs could provide inventive solutions to a range of biomedical issues and enhance human health.

2 Nanocomposites Based on Quantum Dots

The utilisation of nanocomposites that are founded on quantum dots (QDs) has garnered a growing amount of interest in contemporary times, owing to their exceptional optical and electronic characteristics, as well as their potential utility in diverse domains such as energy, electronics, and biomedicine. A nanocomposite refers to a type of material that merges two or more distinct components at the nanoscale to generate a hybrid material that exhibits enhanced properties.

The synthesis of nanocomposites utilising quantum dots (QDs) involves the amalgamation of QDs with diverse materials, including polymers, metals, and inorganic nanoparticles. The special properties and their applications are tabulated and summarized in Table 1. The nanocomposites exhibit distinctive characteristics that arise from the amalgamation of the properties of the quantum dots and the other constituent materials. These properties can be customised by manipulating the composition and structure of the nanocomposite. QD-polymer composites are among the most prevalent forms of nanocomposites that incorporate quantum dots. Quantum dots (QDs) have the capability to be incorporated into diverse polymer matrices, including polystyrene, poly(methyl methacrylate), and polyvinyl alcohol, resulting in the formation of QD-polymer nanocomposites. The nanocomposites have demonstrated superior optical characteristics, including heightened fluorescence intensity and enhanced stability in comparison to unbound quantum dots. The integration of QD-polymer nanocomposites exhibits promising prospects in various fields such as optical sensors, photovoltaic devices, and biological imaging.

QD-metal nanocomposites represent an additional category of nanocomposites that incorporate quantum dots. Quantum dots (QDs) have the potential to form nanocomposites with metals, including gold and silver. The nanocomposites exhibit distinctive plasmonic characteristics that render them suitable for employment in sensing and imaging applications. The utilisation of QD-metal nanocomposites has

Table 1 Various parameters for evaluating the role and features of QDs based nanocomposites

Categories	Quantum dot-based nanocomposites
Classification	
Composition	Core–shell, alloyed, heterostructure
Dimensionality	0D, 1D, 2D, 3D
Design	
Surface modification	Ligand exchange, surface functionalization
Morphology	Nanoparticles, nanowires, nanosheets, nanorods, nanotubes
Fabrication	
Synthesis	Chemical, physical, biological
Assembly	Self-assembly, Co-assembly, Template-assisted assembly
Applications	
Optoelectronics	LEDs, solar cells, display devices, photodetectors, photoelectrochemical cells
Biomedical	Imaging, drug delivery, biosensing, photodynamic therapy
Energy storage	Batteries, supercapacitors, fuel cells
Catalysis	Photocatalysis, electrocatalysis, biocatalysis
Cytotoxicity	
In vitro assays	MTT assay, LDH assay, ROS assay, cell viability assay
In vivo studies	Acute and chronic toxicity studies, biodistribution studies, histopathology, immunotoxicity studies

been demonstrated to manifest escalated catalytic efficacy in contrast to unbound QDs, thereby presenting a potential avenue for their application in photocatalytic contexts.

QD-inorganic nanoparticle nanocomposites can be generated by incorporating inorganic nanoparticles, such as silica and iron oxide, with quantum dots. The nanocomposites exhibit promising prospects for utilisation in drug delivery and magnetic resonance imaging. The utilisation of QD-inorganic nanoparticle nanocomposites has demonstrated enhanced biocompatibility and stability in contrast to unbound QDs, rendering them a propitious candidate for employment in biomedical domains. Quantum dot (QD) nanocomposites have been studied for their potential application in energy-related fields, including solar cells and light-emitting diodes (LEDs). Quantum dots (QDs) have the potential to be integrated into a range of materials, including perovskites and graphene, for the purpose of generating QD-energy nanocomposites. The nanocomposites have demonstrated superior light harvesting and charge transport characteristics in comparison to conventional materials, rendering them a viable candidate for deployment in future energy devices.

Nanocomposites based on quantum dots (QDs) exhibit distinctive characteristics that render them highly favourable for a diverse range of applications in the fields of energy, electronics, and biomedicine. The versatility and adaptability of QD-based nanocomposites can be attributed to the ability to modify their properties by manipulating the composition and structure of the nanocomposite. Additional investigation is required to comprehensively comprehend the potential of nanocomposites based on quantum dots and to enhance their characteristics for particular uses.

Nanocomposites that utilise quantum dots as their building blocks can be categorised according to various factors, such as their composition, dimensionality, surface modification, morphology, and methods of fabrication. Quantum dots of core–shell, alloyed, and heterostructure types can be produced in configurations of 0D, 1D, 2D, or 3D, accompanied by diverse surface modifications and morphologies. Various fabrication techniques can be employed, such as chemical, physical, or biological synthesis methods, in addition to self-assembly, co-assembly, and template-assisted assembly. Quantum dot-based nanocomposites have a wide range of potential applications, including but not limited to optoelectronics, biomedical, energy storage, and catalysis. The utilisation of quantum dots in optoelectronic devices such as light-emitting diodes, solar cells, display technologies, photodetectors, and photoelectrochemical cells is a prevalent area of research and development. Quantum dots have potential applications in the biomedical field, including but not limited to imaging, drug delivery, biosensing, and photodynamic therapy. Quantum dots have been employed in energy storage applications such as batteries, supercapacitors, and fuel cells. Additionally, catalysis applications have utilised quantum dots in photocatalysis, electrocatalysis, and biocatalysis. The evaluation of quantum dot-based nanocomposites' safety necessitates the conduct of cytotoxicity investigations. The toxicity of quantum dots on cultured cells can be assessed through in vitro assays, including but not limited to the MTT assay, LDH assay, ROS assay, and cell

viability assay. Quantum dots' toxicity in living organisms can be evaluated through in vivo studies, including acute and chronic toxicity studies, biodistribution studies, histopathology, and immunotoxicity studies.

3 Cytotoxicity of Quantum Dots

Quantum dots (QDs) are diminutive particles, measuring in the nanometer range, that are composed of semiconducting materials. Their distinctive optical characteristics, such as size-dependent fluorescence and exceptional photostability, have rendered them extensively employed in biological investigations and medical imaging [32]. Notwithstanding their potential utility in biological and medical applications, concerns have been raised regarding their cytotoxicity, which may constrain their usage. Cytotoxicity pertains to the capacity of a substance to induce harm or mortality to cellular entities. QDs can undergo diverse mechanisms that lead to their toxicity, such as oxidative stress, cellular membrane disruption, and DNA damage [33]. The likelihood of cytotoxicity is contingent upon various factors, such as the dimensions, surface properties, and constitution of the quantum dots. The determination of cytotoxicity of quantum dots is significantly influenced by their size. QDs with a diameter smaller than 10 nm have been observed to exhibit greater toxicity compared to their larger counterparts. This is attributed to their higher probability of penetrating cell membranes and interacting with cellular components. Furthermore, the toxicity of quantum dots (QDs) can be significantly influenced by their surface chemistry. It has been demonstrated that the cytotoxicity of quantum dots (QDs) can be mitigated by coating them with hydrophilic molecules, such as polyethylene glycol (PEG), in contrast to QDs that are uncoated or coated with hydrophobic molecules [34]. The cytotoxicity of quantum dots (QDs) can be influenced by their composition. Cadmium-based quantum dots (QDs) are frequently employed owing to their robust fluorescence characteristics; however, they have demonstrated significant cytotoxicity towards cells. Cadmium is a heavy metal that possesses toxic properties and has the potential to induce oxidative stress and DNA damage [35]. Consequently, scholars have endeavoured to fabricate substitute quantum dot (QD) materials with reduced toxicity, including zinc-based QDs or QDs composed of non-toxic elements such as silicon. Several assays have been devised to evaluate the cytotoxicity of quantum dots (QDs). The aforementioned assays encompass techniques for evaluating cell viability, membrane integrity, reactive oxygen species (ROS) production, and DNA harm. Broadly speaking, the assays conducted have demonstrated that quantum dots (QDs) may exhibit cytotoxicity towards cells when present in elevated concentrations or in specific environments. The precise threshold for cytotoxicity may exhibit variability contingent upon the specific quantum dot (QD) variant and the particular cell type under investigation. Although QDs possess the potential for cytotoxicity, they remain a subject of interest for various biological and medical applications [36]. Scientists are currently engaged in the development of quantum dots that exhibit reduced toxicity, while retaining their distinctive optical characteristics.

Furthermore, endeavours are underway to enhance comprehension of the underlying mechanisms of quantum dot (QD) cytotoxicity [37]. This could facilitate the creation of safer QD materials and enhance their utility in biological and medical research. The matter of quantum dots' cytotoxicity is multifaceted and contingent upon various variables such as their dimensions, surface characteristics, and formation. Although quantum dots (QDs) possess the potential to induce cellular toxicity, they exhibit distinctive optical characteristics that render them highly advantageous for a diverse array of applications. Further investigation is required to enhance comprehension of the underlying mechanisms responsible for the cytotoxicity of quantum dots (QDs) and to formulate QD materials that are safer for application in biological and medical research.

4 Mechanisms of Cytotoxicity of Quantum Dots

Early reports talked about successes in single-molecule detection [38], single-cell tracking [39], and whole embryo or animal in vivo imaging. However, recent publications have brought up the need for a serious assessment of QDs' potential long-term cytotoxicity [33]. The research conducted on cadmium (Cd) chalcogenide materials, specifically those with CdS, CdSe, or CdTe cores, with or without a ZnS shell, has demonstrated that QDs exhibit cytotoxicity when present in micromolar concentrations in the growth medium [40]. Furthermore, the studies have revealed that the toxicity of QDs is positively correlated with the exposure of the core surface cadmium atoms to the surrounding medium. The investigation of the cytotoxicity of CdSe and CdSe/ZnS nanoparticles has been conducted with varying surface modifications, including mercaptopropionic acid coating, silanization, and polymer coating (Fig. 1). Quantitative values pertaining to the initiation of cytotoxic effects in culture media devoid of serum are provided for all instances. According to Kirchner et al. the viability of cells is reduced at elevated concentrations of nanoparticles, regardless of their type. Hence, it is plausible that diverse mechanisms of cytotoxicity could be operational (Fig. 2).

5 Factors Influencing Cytotoxicity of Quantum Dots

The potential for quantum dots (QDs) to exhibit cytotoxicity is contingent upon various factors, such as their size, shape, surface chemistry, composition, concentration, exposure duration, and the specific type of cell under consideration.

The dimension of quantum dots (QDs) is a crucial determinant in assessing their potential cytotoxicity. Quantum dots (QDs) that possess a diameter of less than 10 nm exhibit higher toxicity compared to their larger counterparts due to their ability to readily permeate cell membranes and interact with intracellular constituents. The diminutive dimensions of quantum dots (QDs) result in an augmented surface area

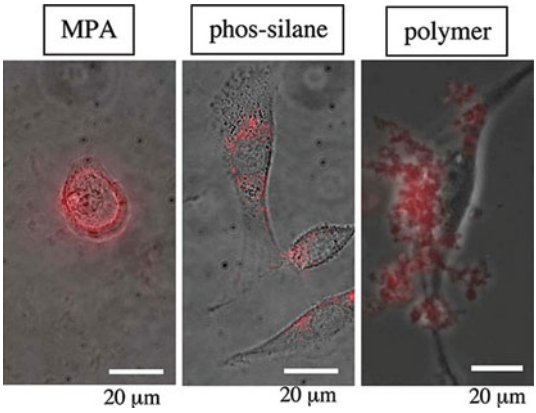

Fig. 1 Different type of nanoparticles and their cellular uptake. Reproduced with the permission from ref. [40] © 2005 American chemical society

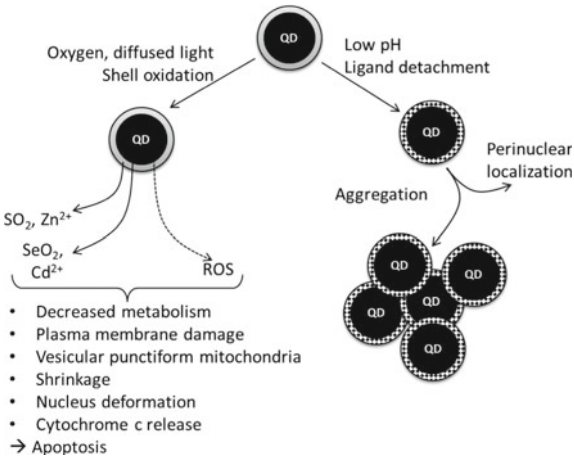

Fig. 2 Illustrations depicting the various mechanisms leading to the cytotoxicity of quantum dots

to volume ratio, thereby enhancing their reactivity and ability to generate reactive oxygen species (ROS). Furthermore, diminutive quantum dots exhibit a higher propensity to interact with proteins and nucleic acids, thereby inducing modifications in cellular activity and DNA impairment.

The cytotoxicity of quantum dots can also be influenced by their shape. The cytotoxicity of quantum dots (QDs) exhibiting distinct morphologies, including spherical, rod-shaped, and tetrapod-shaped, has been the subject of investigation. Several research studies have indicated that quantum dots (QDs) with a spherical shape exhibit lower levels of toxicity as compared to those with a rod or tetrapod morphology. One possible explanation for this phenomenon is that quantum dots (QDs) exhibiting a rod or tetrapod morphology possess a greater surface area, thereby facilitating heightened cellular interactions.

The cytotoxicity of quantum dots (QDs) is significantly influenced by their surface chemistry. The surface chemistry of QDs can be modified through the use of

diverse molecules, including PEG, mercaptoundecanoic acid, or amines. Quantum dots (QDs) that are coated with hydrophilic molecules, such as polyethylene glycol (PEG), have demonstrated a decrease in cytotoxicity when compared to QDs that are uncoated or coated with hydrophobic molecules. The application of the coating has the potential to impede the aggregation of quantum dots (QDs) and subsequently reduce the probability of cellular interaction.

The cytotoxicity of quantum dots (QDs) can be influenced by their composition. Cadmium-based quantum dots (QDs) are frequently utilised in view of their robust fluorescence characteristics; however, they have been demonstrated to exhibit significant cytotoxicity towards cells. Cadmium is a heavy metal that possesses toxic properties and has the potential to induce oxidative stress and DNA damage. Consequently, scholars have devised substitute quantum dot (QD) substances, such as zinc-based QDs or QDs composed of innocuous elements like silicon, with the aim of mitigating toxicity.

The concentration of quantum dots (QDs) is a crucial element that significantly impacts their cytotoxicity. Elevated concentrations of quantum dots (QDs) may result in heightened toxicity due to the augmented probability of interaction with cellular constituents. At elevated concentrations, quantum dots (QDs) have been observed to induce apoptosis, resulting in cellular demise, through the disruption of cellular membranes.

The cytotoxicity of quantum dots can be influenced by the duration of exposure. Extended exposure to quantum dots (QDs) may result in heightened toxicity due to their potential to accumulate within cells and induce harm. If the concentration of QDs is sufficiently high, short-term exposure can result in toxicity. When evaluating the cytotoxicity of QDs, it is important to take into account the duration of exposure.

Cellular type plays a significant role in determining the cytotoxicity of quantum dots (QDs). Certain cellular varieties exhibit greater susceptibility to the toxicity of quantum dots than others. As an illustration, quantum dots (QDs) have demonstrated a greater cytotoxicity towards cancerous cells in comparison to healthy cells. Furthermore, the cellular attributes, such as their metabolic functionality and transporter expression, can impact their engagement with quantum dots and ascertain their cytotoxicity.

To summarise, the matter of QD cytotoxicity is multifaceted and can be impacted by various factors such as size, shape, surface chemistry, composition, concentration, exposure duration, and cellular classification. Comprehending these factors is of utmost importance for the secure and efficient utilisation of quantum dots (QDs) in the realm of biological and biomedical investigation. Additional investigation is required to gain a complete understanding of the mechanisms that underlie the cytotoxicity of quantum dots and to devise QD-based applications that are both safer and more efficacious.

6 In Vitro and in Vivo Assays for Evaluating Cytotoxicity of Quantum Dots

The evaluation of the cytotoxicity of quantum dots (QDs) is a crucial stage in their advancement for biomedical purposes (Table 2). Quantum dots (QDs) possess distinctive optical and electronic characteristics, rendering them highly desirable for a range of biomedical purposes, including but not limited to imaging, sensing, and drug delivery. Nevertheless, the utilisation of Quantum Dots (QDs) has been linked to probable toxicity owing to their diminutive dimensions, elevated surface area, and capability to produce reactive oxygen species (ROS). Hence, it is imperative to conduct a thorough evaluation of the cytotoxicity of quantum dots to ensure their safe and efficient utilisation as nanomaterials. The evaluation of QDs cytotoxicity is commonly performed through two primary assay types, namely in vitro assays and in vivo assays. Cell culture models are utilised for in vitro assays, whereas animal models are employed for in vivo assays. The selection of assay is contingent upon the particular research inquiry and developmental phase, as both in vitro and in vivo assays possess their respective merits and drawbacks. The simplicity, speed, and cost-effectiveness of in vitro assays make them a popular choice for the preliminary screening of quantum dot (QD) cytotoxicity. The MTT assay, LDH assay, ROS assay, and flow cytometry are among the frequently employed in vitro assays for evaluating the cytotoxicity of quantum dots.

The MTT assay is a widely used colorimetric technique that quantifies the metabolic activity of cells. The efficacy of the assay is predicated upon the capacity of living cells to convert the tetrazolium salt MTT, which is yellow in colour, to a purple formazan product. The quantity of formazan generated exhibits a direct correlation with the count of viable cells. The present assay is characterised by its straightforwardness and extensive application, owing to its capability to process a large number of samples and furnish numerical results. The MTT assay's reliability in determining cell viability may be compromised due to potential interference from QDs. The LDH assay is a biochemical technique utilised for quantifying the liberation of lactate dehydrogenase (LDH) from cells that have undergone damage. The assay evaluates cellular demise as opposed to vitality and is a straightforward and extensively employed technique. Nevertheless, the method's ability to differentiate between necrosis and apoptosis may be limited, and it could be susceptible to QD interference.

The quantification of reactive oxygen species (ROS) within cells is achieved through employment of the ROS assay. The aforementioned assay furnishes data pertaining to oxidative stress and plausible mechanisms underlying the cytotoxicity of quantum dots. The assessment of reactive oxygen species (ROS) may not provide a direct indication of cellular viability and could potentially be impacted by interference from quantum dots (QDs).

Flow cytometry is a robust technique that enables the quantification of cell viability, apoptosis, and cell cycle. Flow cytometry is a valuable tool for investigating various cellular parameters and can be employed to examine the impact of

Table 2 Various assays adopted to justify cytotoxicity of QDs based nanocomposites

Assay	Description	Advantages	Limitations
In vitro assays			
MTT assay	Measures metabolic activity of cells	Simple and widely used; High-throughput; Provides quantitative data	May not accurately reflect cell viability; Can be influenced by QD interference
LDH assay	Measures release of lactate dehydrogenase from damaged cells	Measures cell death rather than viability; Simple and widely used	May not distinguish between necrosis and apoptosis; Can be influenced by QD interference
ROS assay	Measures reactive oxygen species levels in cells	Provides information on oxidative stress and potential mechanism of cytotoxicity	May not directly reflect cell viability; Can be influenced by QD interference
Flow cytometry	Measures cell viability, apoptosis, and cell cycle	Provides detailed information on multiple cellular parameters	Requires specialized equipment and expertise
In vivo assays			
Histology	Examines tissue structure and cellular morphology	Provides information on tissue damage and inflammation	May not accurately reflect systemic toxicity; Requires tissue sampling
Biodistribution studies	Measures distribution and accumulation of QDs in tissues	Provides information on QD pharmacokinetics	May not accurately reflect toxicity; Requires specialized equipment and expertise
Blood chemistry analysis	Measures levels of enzymes, electrolytes, and other biomarkers in blood	Provides information on systemic toxicity and organ function	May not accurately reflect QD toxicity; Requires blood sampling
Behavioral assays	Measures changes in behavior or motor function	Provides information on potential neurological effects of QDs	May not directly reflect QD toxicity; Requires specialized equipment and expertise

quantum dots (QDs) on particular cell populations. Nevertheless, the utilisation of flow cytometry necessitates specialised equipment and expertise.

The utilisation of in vivo assays is crucial in the assessment of the systemic toxicity of quantum dots (QDs) and their capacity to accumulate in various tissues and organs. The assessment of quantum dot (QD) cytotoxicity in vivo typically involves a range of assays, including histology, biodistribution studies, blood chemistry analysis, and behavioural assays.

Histology pertains to the analysis of the structural composition of tissues and the morphology of cells. The utilisation of histological analysis enables the acquisition of valuable insights regarding tissue damage and inflammation, thereby facilitating the evaluation of the impact of QDs on various organs. Nevertheless, it should be noted that histology may not provide a precise representation of systemic toxicity and necessitates the collection of tissue samples.

Biodistribution investigations encompass the assessment of quantum dot (QD) dispersion and aggregation across diverse tissues and organs. Biodistribution investigations furnish insights into the pharmacokinetics of quantum dots (QDs) and serve as a means to evaluate the likelihood of QD accumulation in non-targeted anatomical sites. Notwithstanding, biodistribution investigations may not precisely mirror toxicity and necessitate specialised equipment and expertise.

The process of blood chemistry analysis entails the quantification of various biomolecules such as enzymes, electrolytes, and other biomarkers present in the blood. The analysis of blood chemistry offers valuable insights into the toxicity of a substance on the entire system and the functioning of organs. This method can be employed to evaluate the impact of quantum dots (QDs) on various organs. Notwithstanding, the toxicity of QD may not be precisely reflected by means of blood chemistry analysis and necessitates the collection of blood samples.

Behavioural assays encompass the quantification of alterations in animal conduct in reaction to exposure to QD. Behavioural assays have the capacity to furnish insights into the plausible impacts of quantum dots (QDs) on neurological function. Moreover, they can be employed to evaluate the likelihood of QDs to traverse the blood–brain barrier. Notwithstanding, behavioural assays are susceptible to various factors, including stress and environmental conditions.

The evaluation of quantum dots' cytotoxicity necessitates the utilisation of both in vitro and in vivo assays. The application of in vitro assays is advantageous for the preliminary assessment of cytotoxicity associated with QDs. Conversely, in vivo assays are indispensable for the comprehensive evaluation of systemic toxicity and the capacity of QDs to accumulate in diverse tissues and organs. The selection of an appropriate assay is contingent upon the particular research inquiry and developmental phase, and a comprehensive evaluation of quantum dot cytotoxicity may require the use of multiple assays.

7 Cytotoxicity of Quantum Dots Based Nanocomposites

The potential cytotoxicity of quantum dots (QDs) and QD-based nanocomposites has been a significant concern, particularly in the field of biomedical applications. The term cytotoxicity pertains to the capacity of a substance to induce harm or mortality to cells. The degree of cytotoxicity exhibited by QDs is predominantly influenced by factors such as their size, shape, surface chemistry, and composition. The diminutive dimensions of quantum dots (QDs) facilitate their cellular membrane penetration and subsequent interaction with intracellular constituents, potentially resulting in

cytotoxicity. Research has demonstrated that quantum dots (QDs) have the potential to trigger oxidative stress, DNA harm, and apoptosis (a process of programmed cell death) within cellular structures. The potential for QDs to induce cell death is contingent upon the specific cellular population under investigation. As demonstrated by previous studies, quantum dots (QDs) have exhibited a greater propensity to elicit cytotoxic responses in malignant cells compared to their non-cancerous counterparts.

In order to address the issue of cytotoxicity associated with QDs, scholars have devised nanocomposites that are based on QDs and contain biocompatible substances, such as lipids and polymers. The utilisation of nanocomposites has been shown to enhance the stability, solubility, and biocompatibility of quantum dots (QDs), thereby rendering them more appropriate for employment in biomedical contexts. A research study was conducted to examine the cytotoxic properties of nanocomposites based on quantum dots (QDs), which were composed of zinc sulphide (ZnS)-coated QDs and polyethylene glycol (PEG). According to the research, the utilisation of PEG coating resulted in a notable decrease in the cytotoxic effects of QDs on human hepatoma cells, as indicated by the measurements of cell viability and lactate dehydrogenase (LDH) release. A further investigation was conducted to examine the cytotoxic properties of nanocomposites based on quantum dots (QDs), which were comprised of chitosan and hyaluronic acid coatings. According to the research, the application of chitosan coating resulted in enhanced stability and biocompatibility of quantum dots (QDs) in human breast cancer cells. The assessment was carried out by measuring cell viability and apoptosis. Nevertheless, the cytotoxicity associated with nanocomposites based on quantum dots remains a subject of ongoing investigation, and additional research is required to comprehensively comprehend the potential advantages and disadvantages of these substances. Furthermore, the comprehensive understanding of the enduring impacts of exposure to QD on human health remains incomplete, necessitating further investigation in this domain. The matter of cytotoxicity pertaining to QDs and QD-based nanocomposites is multifaceted and contingent upon several variables, such as dimensions, morphology, surface properties, and constitution. Although quantum dots (QDs) have demonstrated potential in various biomedical applications, it is crucial to meticulously assess and alleviate their potential cytotoxic impacts. Further investigation is required to comprehensively comprehend the potential advantages and drawbacks of biocompatible nanocomposites based on QDs, which could potentially mitigate the cytotoxicity of these substances.

8 Mechanisms of Cytotoxicity of Quantum Dots Based Nanocomposites

The matter of cytotoxicity pertaining to quantum dots (QDs) and QD-based nanocomposites is multifaceted and encompasses a range of mechanisms. The distinctive characteristics and diminutive dimensions of quantum dots (QDs) enable them to engage

with intracellular constituents, thereby potentially engendering cytotoxic outcomes. The cytotoxicity of QDs and QD-based nanocomposites is attributed to several mechanisms, including oxidative stress, DNA damage, and apoptosis. The phenomenon of oxidative stress arises due to a state of disequilibrium between the generation of reactive oxygen species (ROS) and the capacity of cells to eliminate them (Fig. 3). Quantum dots (QDs) have the ability to produce reactive oxygen species (ROS) through various mechanisms such as surface defects, photoactivation, and electron transfer. The surplus of reactive oxygen species (ROS) has the potential to inflict harm upon the cellular constituents, including lipids, proteins, and DNA, thereby resulting in cytotoxic consequences. The cytotoxicity of quantum dots (QDs) and QD-based nanocomposites can be attributed to DNA damage as a mechanism. Quantum dots (QDs) have the capability to interact directly with DNA, which can result in the occurrence of single-strand breaks, double-strand breaks, and oxidative damage. DNA damage has the potential to trigger various cellular pathways, including the DNA damage response and p53 pathway, which may result in cell cycle arrest or apoptosis. The process of apoptosis is a predetermined cellular death pathway that is triggered as a result of internal or external cellular stressors. Quantum dots (QDs) have the ability to initiate the intrinsic apoptotic pathway through the disruption of the mitochondrial membrane potential, which results in the release of cytochrome c into the cytoplasm. Cytochrome c has the ability to initiate the activation of caspase enzymes, which in turn can induce the fragmentation of intracellular constituents, ultimately resulting in apoptosis.

The cytotoxicity of quantum dots (QDs) and QD-based nanocomposites is contingent upon the cell type that is subjected to them, in addition to the aforementioned mechanisms. As demonstrated by previous studies, quantum dots (QDs) have exhibited a greater propensity to elicit cytotoxic responses in malignant cells as compared to their non-malignant counterparts. The observed phenomenon could potentially be attributed to variances in cellular absorption, metabolic processes, and reactivity to oxidative stress. In order to address the issue of cytotoxicity associated with QDs and QD-based nanocomposites, scholars have devised biocompatible coatings, including polymers and lipids. The application of coatings on quantum dots (QDs) has been shown to enhance their stability, solubility, and biocompatibility, thereby mitigating their cytotoxicity. In general, the matter of cytotoxicity pertaining to quantum dots (QDs) and QD-based nanocomposites is multifaceted and encompasses a range of mechanisms. The diminutive dimensions and distinctive characteristics of quantum dots (QDs) may give rise to cellular oxidative stress, DNA harm, and programmed cell death. The creation of biocompatible coatings presents a potential solution for mitigating the cytotoxicity of said materials, thereby rendering them more appropriate for use in biomedical contexts.

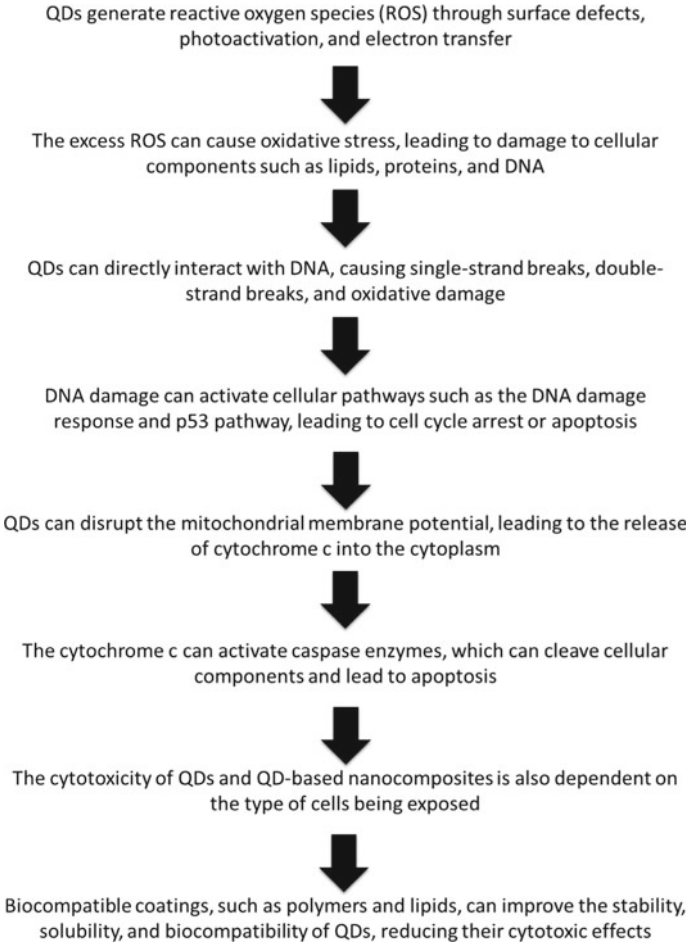

Fig. 3 Flow diagram of the consecutive steps of the mechanism of cytotoxicity of QDs based nanocomposites

9 Cytotoxicity for Polymer Encapsulated QDs Systems

Polymer-encapsulated quantum dot (QD) systems refer to composite materials that consist of QDs and a polymeric matrix that envelops and encapsulates the QDs. The polymeric matrix confers stability and safeguarding to the quantum dots, while simultaneously endowing distinctive characteristics to the composite material. The utilisation of polymer-encapsulated quantum dot (QD) systems has garnered considerable interest owing to their prospective applications in various fields including sensing, imaging, and drug delivery. The incorporation of quantum dots (QDs) into a polymer matrix through encapsulation confers numerous benefits compared to the utilisation of uncoated QDs. Encapsulation is a technique that enhances the stability

of quantum dots (QDs) by safeguarding them against various environmental factors, including moisture, heat, and light, which may cause degradation of the QDs over time. The utilisation of encapsulation also facilitates the enhancement of biocompatibility of quantum dots (QDs), a crucial aspect for biomedical purposes. The composite material can be endowed with specific functionality by modifying the polymeric matrix, for instance, to target particular cells or tissues. The categorization of polymer encapsulated quantum dot (QD) systems is contingent upon the specific polymer employed during their synthesis. Polyethylene glycol (PEG), polystyrene (PS), polyvinyl alcohol (PVA), and polyacrylamide (PAA) are among the frequently employed polymers for the purpose of encapsulating quantum dots (QDs). Polymers exhibit distinct characteristics that can be customised to fulfil particular application demands.

Polyethylene glycol (PEG)-based polymer encapsulated quantum dot (QD) systems have demonstrated biocompatibility and have been extensively employed in biomedical fields, including imaging and drug delivery. Polyethylene glycol (PEG) can be tailored to confer distinct functionalities to the composite material, for instance, by selectively targeting particular cells or tissues. The utilisation of PEG-based polymer encapsulated quantum dots (QDs) has demonstrated notable biocompatibility and robustness, rendering them a promising candidate for employment in the field of biomedicine. Polymer-encapsulated quantum dot (QD) systems based on polystyrene (PS) have been investigated for various purposes, including sensing and imaging. Polystyrene (PS) is a polymer that exhibits hydrophobic properties, rendering it a viable option for sensing applications. Moreover, the surface chemistry of PS can be tailored to confer distinct functionalities to the composite material. The potential toxicity of PS-based polymer encapsulated QDs warrants careful evaluation due to the hydrophobic properties of the polymer, which may result in cellular uptake and subsequent toxicity. The utilisation of PVA-based polymer encapsulated QDs systems has been explored in various fields, including biosensors and imaging. Polyvinyl alcohol (PVA) is a polymer that is both biocompatible and water-soluble, rendering it appropriate for use in various biomedical applications. The optimisation of composite material properties can be achieved through the tuning of quantum dot size and surface chemistry. The utilisation of PAA-based polymer encapsulated QDs systems has been explored for various purposes, including biosensing and imaging. Polyacrylic acid (PAA) is a hydrophilic polymer that exhibits solubility in water and can be conveniently tailored to confer distinct attributes to the resulting composite material. The properties of the composite material can be adjusted by altering the surface chemistry of the PAA. The cytotoxicity mechanism of polymer-encapsulated quantum dot (QD) systems is contingent upon various factors, including the polymer type, QD size and surface chemistry, and composite material concentration. The deleterious effects of polymer encapsulated quantum dot (QD) systems on cells can be ascribed to multiple factors, such as oxidative stress, DNA damage, and apoptosis. It is imperative to conduct a thorough assessment of the toxicity of polymer-encapsulated quantum dot (QD) systems prior to their implementation in biomedical contexts, given the potential for significant variability in toxicity levels across different composite materials.

Polymeric matrices comprising natural polymers like chitosan, gelatin, and alginate have been employed as hosts for polymer nanocomposites containing quantum dots. Chitosan is a positively charged polysaccharide that originates from chitin, a readily available substance found in the shells of crustaceans. Gelatin is a biopolymer that is acquired through the process of partial hydrolysis of collagen, while alginate is a linear polysaccharide that originates from seaweed. The biocompatibility, biodegradability, and low toxicity of these polymers render them highly suitable for employment in biomedical contexts. The investigation of the cytotoxicity of polymer nanocomposites based on quantum dots in natural polymer systems has been conducted across multiple cell lines. Research findings indicate that QD nanocomposites based on chitosan demonstrate biocompatibility and low toxicity. The size of quantum dots (QDs) has been identified as a contributing factor to the cytotoxicity of chitosan-based QD nanocomposites, whereby larger QDs are more toxic than their smaller counterparts. Likewise, it has been demonstrated that QD nanocomposites based on gelatin manifest reduced cytotoxicity and are deemed appropriate for employment in the realm of biomedicine. Research has also explored nanocomposites consisting of alginate and quantum dots, which have demonstrated minimal cytotoxicity.

10 Strategies for Reducing Cytotoxicity of Quantum Dots Based Nanocomposites

Nanocomposites utilising quantum dots (QDs) have demonstrated significant promise for a range of biomedical purposes, including drug delivery and bioimaging. Nevertheless, the cytotoxicity of these substances remains a significant issue, impeding their extensive application. Various approaches have been investigated to address this concern, such as surface modification, encapsulation, and size and morphology optimisation. The objective of these methodologies is to mitigate the toxicity associated with nanocomposites based on quantum dots, while simultaneously preserving their intended characteristics.

11 Surface Functionalization

The implementation of surface functionalization has proven to be a viable strategy in mitigating the cytotoxic effects of nanocomposites that utilise quantum dots (QDs). Several techniques for surface functionalization have been devised to alter the surface characteristics of quantum dots (QDs) and enhance their biocompatibility. Several surface functionalization strategies have been utilised to mitigate the cytotoxicity of nanocomposites based on quantum dots (QDs).

The application of biocompatible polymers as a coating for quantum dots (QDs) has been demonstrated to effectively mitigate their cytotoxicity. Polyethylene glycol (PEG) is a commonly utilised biocompatible polymer for this purpose. The process of PEGylation enhances the hydrophilic nature of the surface of quantum dots (QDs), thereby impeding the binding of biomolecules and diminishing their interaction with cells, ultimately leading to an enhancement in biocompatibility.

The enhancement of biocompatibility of quantum dots (QDs) through surface modification with amino acids and peptides has been established. As an illustration, the surface conjugation of arginine onto quantum dots (QDs) has been shown to enhance their cellular uptake, whereas the conjugation of cysteine has been demonstrated to mitigate their cytotoxicity.

The process of conjugation with targeting ligands involves the binding of quantum dots (QDs) with specific targeting ligands, such as antibodies or aptamers. This conjugation process can enhance the specificity of QDs towards a particular cell type, thereby reducing their cytotoxicity. The utilisation of this methodology facilitates the targeted interaction of quantum dots (QDs) with specific cells, thereby reducing their interaction with non-targeted cells.

The utilisation of zwitterionic materials has been demonstrated to enhance the biocompatibility of nanocomposites based on quantum dots (QDs). These materials possess surface charges that are both positively and negatively charged. The surface charges of these materials confer upon them the ability to resist non-specific binding and minimise their interaction with cells.

The process of conjugation with antioxidant molecules has been observed to reduce the toxicity of quantum dots (QDs). This is achieved through the binding of QDs with antioxidant molecules such as glutathione or ascorbic acid, which effectively scavenge reactive oxygen species (ROS) and mitigate the oxidative stress induced by QDs.

The application of bioactive molecules for surface modification has been demonstrated to effectively mitigate the cytotoxicity of quantum dots (QDs). Specifically, enzymes or peptides have been utilised as bioactive molecules to modify the surface of QDs. The conjugation of catalase to quantum dots (QDs) has been observed to decrease the production of reactive oxygen species (ROS). Conversely, the conjugation of cell-penetrating peptides has been shown to augment the cellular uptake of QDs and mitigate their toxicity.

The utilisation of natural polymers, such as chitosan or alginate, for the purpose of surface modification of quantum dots (QDs) has been shown to enhance their biocompatibility and mitigate their toxicity. The innate biocompatibility and facile biodegradability of these natural polymers render them suitable contenders for the purpose of surface functionalization of quantum dots.

The biocompatibility of quantum dots (QDs) can be enhanced and their toxicity reduced through conjugation with non-toxic nanoparticles, such as gold or silver nanoparticles. Conjugated nanoparticles may be subjected to functionalization with targeting ligands, thereby augmenting their specificity towards a specific cell type.

12 Encapsulation of Quantum Dots

The process of encapsulating quantum dots (QDs) is a versatile approach that has the potential to enhance their biocompatibility and mitigate their cytotoxicity. The proposed methodology entails the incorporation of quantum dots (QDs) into a safeguarding matrix, resulting in the formation of core–shell architecture. The implementation of a protective matrix can serve as a physical impediment to hinder the release of hazardous quantum dots (QDs) into the surroundings. Additionally, it can enhance the durability and suitability of QDs for biological applications.

The utilisation of polymeric encapsulation is a widely employed approach in mitigating the cytotoxic effects of nanocomposites based on quantum dots. Polymeric materials, including PLGA, PEG, and PEI, are frequently employed for the encapsulation of QDs owing to their biocompatible nature, minimal toxicity, and adaptability. The utilisation of polymeric encapsulation presents a viable means of establishing a stable and safeguarding coating around quantum dots (QDs), thereby impeding their direct interaction with cells and reducing their potential for cytotoxicity.

The utilisation of lipid-based encapsulation has been implemented as an alternative strategy to mitigate the cytotoxic effects of nanocomposites based on quantum dots. The proposed methodology involves the encapsulation of quantum dots (QDs) within a lipid bilayer, resulting in the formation of a structure similar to a liposome. The lipid bilayer serves as a physical barrier that can hinder the direct interaction between quantum dots (QDs) and cells, thus mitigating their cytotoxic effects. The utilisation of lipid-based encapsulation presents an additional benefit of emulating the cellular biological membranes, thereby enhancing the cellular absorption and transportation of quantum dots (QDs).

The implementation of silica-based encapsulation has been utilised as a third approach in mitigating the cytotoxicity associated with nanocomposites based on quantum dots (QDs). The proposed methodology involves the integration of quantum dots (QDs) into a silica matrix, resulting in the formation of a core–shell architecture. The utilisation of a silica shell can serve as a physical impediment to impede the release of hazardous quantum dots (QDs) into the surroundings, augment their durability, and elevate their compatibility with biological systems. Studies have demonstrated that the utilisation of silica-based encapsulation can enhance the pharmacokinetic and biodistribution profiles of nanocomposites based on quantum dots (QDs), thereby rendering them more suitable for biomedical applications with improved safety.

The utilisation of protein-based encapsulation has been investigated as a potential strategy to mitigate the cytotoxic effects of nanocomposites based on quantum dots. The proposed methodology involves the encapsulation of quantum dots (QDs) within a protein shell, which may include Bovine Serum Albumin (BSA) or ferritin, resulting in the formation of core–shell architecture. The proteinaceous envelope can serve as a physical barricade, impeding the direct interaction of quantum dots with cellular entities and reducing their potential for cytotoxicity. The utilisation of protein-based encapsulation has demonstrated a notable enhancement in the biocompatibility and

stability of nanocomposites based on quantum dots (QDs). This approach holds great potential as a safe method for the implementation of QDs in biomedical applications.

Various hybrid encapsulation strategies have been devised to mitigate the cytotoxicity of nanocomposites based on quantum dots (QDs) by integrating diverse encapsulation techniques. Core–shell architecture may be established through the process of enclosing quantum dots within a polymeric matrix, followed by the application of a lipid bilayer or silica shell coating. The amalgamation of various encapsulation techniques in hybrid encapsulation can yield synergistic advantages, resulting in enhanced efficacy and safety of nanocomposites based on QDs.

To summarise, encapsulation has shown potential as a viable approach to mitigate the cytotoxic effects of nanocomposites based on quantum dots (QDs). Several encapsulation techniques, including polymeric, lipid-based, silica-based, protein-based, and hybrid encapsulation, can be utilised to reduce the cytotoxicity of nanocomposites based on quantum dots (QDs) and enhance their safety for biomedical purposes. The aforementioned strategies provide flexible alternatives to enhance the biocompatibility and stability of nanocomposites based on quantum dots, thereby facilitating their secure and efficient implementation in diverse biomedical contexts.

13 Coating of Quantum Dots Based Nanocomposites

The application of a coating to quantum dots (QDs) represents a viable approach for mitigating the cytotoxic effects of nanocomposites based on QDs. The proposed methodology entails the application of a biocompatible substance onto the surface of quantum dots (QDs) in order to enhance their stability, biocompatibility, and mitigate their direct cellular interaction.

The implementation of biocompatible polymers, such as polyethylene glycol (PEG) or polyethyleneimine (PEI), to establish a hydrophilic layer on the surface of QDs, is a viable approach to mitigate the cytotoxicity of QDs-based nanocomposites. The utilisation of PEGylation or PEI coating has been observed to impede the direct interaction of quantum dots (QDs) with cells, while simultaneously enhancing their stability, biocompatibility, and mitigating their aggregation in biological surroundings. An additional approach to mitigate the cytotoxicity of nanocomposites based on quantum dots (QDs) involves the application of biomolecules, such as proteins or peptides, to generate a bioactive coating on the QD surface. The utilisation of bioconjugation has been observed to enhance the biocompatibility and specificity of nanocomposites based on quantum dots (QDs), thereby rendering them more suitable for targeted applications. Biomolecules may be chosen to selectively target particular receptors or tissues, thereby augmenting the cellular uptake of quantum dots (QDs) while simultaneously reducing their cytotoxicity. Inorganic coatings, namely silica or metal oxide coatings have been employed as a means to mitigate the cytotoxicity associated with nanocomposites based on quantum dots. The proposed methodology involves the application of a coating of inorganic material onto quantum dots (QDs), which serves as a protective layer. This layer is effective in preventing the

release of hazardous QDs into the surroundings, while also enhancing their stability and biocompatibility. Studies have demonstrated that the application of coatings composed of silica or metal oxide can effectively mitigate the cytotoxicity associated with nanocomposites based on quantum dots (QDs), thereby enhancing their efficacy in the realm of biomedical applications. In addition, the functionalization of quantum dots (QDs) with diminutive molecules, such as enzyme inhibitors or antioxidants, can serve as a coating approach to mitigate their cytotoxic effects. The aforementioned molecules possess the ability to scavenge reactive oxygen species (ROS) or impede enzymes that participate in cellular toxicity. This leads to a decrease in the cytotoxicity of nanocomposites based on quantum dots (QDs) in biological settings.

The application of coating presents a promising approach towards mitigating the cytotoxic effects of nanocomposites based on quantum dots. Several coating techniques, including PEGylation, PEI coating, bioconjugation, inorganic coatings, and functionalization with small molecules, can be utilised to enhance the biocompatibility and stability of nanocomposites based on quantum dots (QDs) and mitigate their cytotoxicity. The implementation of these strategies presents a variety of adaptable solutions for enhancing the safety and effectiveness of nanocomposites based on quantum dots in various biomedical contexts.

14 Conclusion and Future Outlooks

To summarise, the potential cytotoxicity of nanocomposites utilising quantum dots (QDs) is a notable issue that requires resolution prior to their extensive implementation in the field of biomedicine. The distinctive physicochemical characteristics of quantum dots (QDs) that render them appealing for biomedical purposes also render them potentially perilous to both human health and the environment. It is imperative to possess a thorough comprehension of the mechanisms that underlie the cytotoxicity of nanocomposites based on quantum dots (QDs) in order to create QDs-based nanocomposites that are both safe and efficacious for biomedical purposes. Various approaches have been investigated to mitigate the cytotoxic effects of nanocomposites based on quantum dots (QDs), such as surface modification, encapsulation, and coating. The aforementioned methodologies endeavour to enhance the biocompatibility and stability of quantum dots (QDs), while concurrently mitigating their direct interaction with cells, thereby resulting in a reduction of their cytotoxicity. Nevertheless, additional research is imperative to ascertain the most effective coating approach for individual applications and to guarantee the enduring safety of nanocomposites based on quantum dots in vivo.

Prospective directions in this domain encompass the advancement of coating strategies that are both biocompatible and more efficient, the amalgamation of nanocomposites based on quantum dots with other sophisticated materials to augment their performance, and the exploration of their enduring safety and environmental implications. In addition, it is imperative to establish uniform procedures for

assessing the cytotoxicity of nanocomposites based on quantum dots (QDs) and to create regulatory frameworks that ensure their safe application in clinical settings, in order to facilitate their successful integration into medical practise. In general, despite the existing obstacles in ensuring the secure and efficient utilisation of nanocomposites based on quantum dots (QDs), the emergence of inventive approaches to counteract their cytotoxicity presents encouraging prospects for their implementation in various biomedical domains such as diagnosis, imaging, therapy, and drug conveyance.

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Quantum Dots-Based Nanocomposites as Biosensors

Deeksha Thakur, Divya Hudda, Devendra Kumar, Yi-Ting Lai, and Prateek Sharma

Abstract Quantum dots (QD) are inorganic nanoparticles with unique optical and electronic properties, making them attractive candidates for biosensing applications. This chapter discusses the use of QD-based nanocomposites as biosensors for detecting a variety of biomolecules, including proteins, nucleic acids, and small molecules. QD can be functionalized with biomolecules such as antibodies, aptamers, and peptides, allowing for specific binding to target analytes. The resulting QD-biomolecule conjugates can then be incorporated into nanocomposites with other materials such as polymers, metals, or graphene, to enhance the sensitivity, stability, and selectivity of the biosensor. Various detection modalities, including fluorescence, electrochemistry, and surface-enhanced Raman spectroscopy, have been employed to detect the signal generated by QD-based biosensors. The performance of QD-based nanocomposite biosensors has been demonstrated in a wide range of applications, including environmental monitoring, clinical diagnostics, and food safety. However, challenges such as toxicity, stability, and reproducibility still need to be addressed for the practical application of QD-based nanocomposites as biosensors. Overall, the use of QD-based nanocomposites as biosensors shows great promise for the development of highly sensitive and specific biosensing platforms.

Keywords Quantum dots · Biosensors · 2D material · Nanocomposites · QDbased nanocomposites

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1 Introduction

Biosensors have become increasingly important in medical diagnostics due to their ability to provide rapid and accurate detection of various biological analytes. These analytes may include proteins, enzymes, nucleic acids, or metabolites, among others. Biosensors offer numerous advantages over traditional diagnostic methods, including faster detection times, higher sensitivity, specificity, and lower costs. One of the main benefits of biosensors is their ability to detect biomarkers indicative of various diseases, such as cancer, cardiovascular diseases, infectious diseases, and autoimmune disorders. Early detection of these biomarkers can lead to earlier diagnosis and treatment, significantly improving patient outcomes. Biosensors are also useful in monitoring disease progression and treatment efficacy. For example, biosensors can detect changes in the concentration of specific biomarkers over time, allowing clinicians to monitor the effectiveness of treatments and adjust them as needed.

In addition to their use in disease diagnosis and monitoring, biosensors are also being used for point-of-care testing, which enables rapid and on-site diagnosis without the need for specialized equipment or trained personnel. This is particularly important in resource-limited settings, where traditional laboratory-based diagnostic methods may not be feasible. Overall, biosensors have revolutionized medical diagnostics by providing rapid, accurate, and cost-effective detection of biological analytes. Their continued development and application are critical for improving patient outcomes and advancing personalized medicine. Biosensors are also important in environmental monitoring due to their ability to detect and quantify various pollutants and contaminants in air, water, and soil. These pollutants may include heavy metals, pesticides, industrial chemicals, and microbial contaminants, among others. Biosensors offer several advantages over traditional environmental monitoring methods, including their ability to provide real-time, on-site detection with high sensitivity, specificity, and selectivity. This enables rapid and efficient detection of pollutants, allowing for timely and effective interventions to prevent harm to the environment and human health.

Also, to their use in detecting pollutants, biosensors are also useful in monitoring biological processes in the environment, such as the activity of microorganisms and the degradation of pollutants. This can help in understanding the natural processes that occur in the environment and developing strategies for environmental remediation. Biosensors are also being used for the monitoring of food safety, agricultural practices, and the detection of harmful algal blooms in aquatic ecosystems. This allows for identifying potential hazards early on, preventing the spread of contaminated food, and minimizing environmental damage. Overall, biosensors play an important role in environmental monitoring by providing efficient, reliable, and cost-effective detection of pollutants and contaminants. Their continued development and application are critical for improving our understanding of the environment and mitigating the impact of pollutants on human health and the ecosystem.

Biosensors are analytical devices that can detect and measure specific biological or chemical substances in a sample. Nanomaterials have unique physical, chemical,

and biological properties due to their small size and high surface area, which make them ideal for use in biosensors. Nanomaterials have been used to develop biosensors for a wide range of applications, including medical diagnostics, environmental monitoring, food safety, and drug discovery. For instance, biosensors using nanomaterials have been developed for the detection of glucose, cholesterol, and other biomarkers for various diseases such as diabetes and cancer.

Quantum dots (QD) are nanoscale semiconductors with unique physical and chemical properties that distinguish them from bulk materials. The QD possess the properties like size-tunability, high quantum yield, broad absorption spectrum, narrow emission, greater photostability, and high biocompatibility. The electronic and optical properties of QD strongly depend on their size, shape, and composition, which can be precisely controlled during synthesis. This allows for the creation of QD with specific properties that are tailored for various applications.

QD-based nanocomposites have tremendous potential for biosensing applications. Their unique optical properties, high sensitivity, and specificity make them ideal for the detection and analysis of biological molecules, cells, and pathogens.

The small size of QD allows them to penetrate the cell membrane, enabling the detection of intracellular targets with high sensitivity and specificity. The incorporation of QD into a matrix material can further enhance their sensing capabilities, providing a stable and biocompatible platform for biosensing.

One promising application of quantum dots-based nanocomposites in biosensing is the detection of biomolecules, such as proteins and nucleic acids. QD can be functionalized with specific ligands, such as antibodies or aptamers, which bind to the target biomolecule. The binding of the ligand to the target molecule can result in a change in the fluorescence properties of the quantum dot, providing a readout for the presence and concentration of the target biomolecule.

Along with biomolecule detection, quantum dots-based nanocomposites can also be used for the detection of cells and pathogens. The incorporation of QD into a matrix material can enable the fabrication of high-density arrays of sensing elements, providing multiplexed detection capabilities. The high sensitivity and specificity of QD can also enable the detection of low concentrations of cells or pathogens, making them ideal for early disease detection and monitoring. This chapter describes the QD-based nanocomposites for their magnificent properties and their best applications in biosensing in different sectors. The applications have been discussed in detail in further sections.

2 QD for Biosensing

QDs are a type of fluorescent nanomaterial that can emit various wavelengths and are typically composed of atoms from groups II–VI and III–V of the periodic table [1, 2]. Their optoelectronic characteristics are influenced by their shape and size, and their properties are intermediate between those of bulk semiconductors and those of discrete atoms or molecules [3, 4]. QDs are often capped with an inorganic

layer to enhance their quantum yield and signal-to-noise ratio [5]. They have drawn much interest because of their distinctive electronic characteristics and tunable emission spectrum and can be utilized in many different devices and applications, such as solar cells, fluorescent probes, optical switches, and light sources.

QD can undergo modification by surface encapsulation with amphiphilic ligands, salinizing, and exchanging ligands, broadening their potential applications in sensors [6, 7]. QD can be used to create biosensors, allowing researchers to examine cell processes at the molecular level, potentially aiding in the diagnosis and treatment of diseases such as cancer 8. However, the utilization of QDs in biomedical applications is restricted due to the inclusion of toxic heavy metals, such as cadmium, making the development of a nontoxic and biocompatible nanomaterial highly vital [8]. Hybridizing QD with other substances can result in improved resistance to heat and chemicals, increased quantum efficiency, extended durations of excited state, and reduced harmful effects. Biosensors are devices that utilize biological interactions to confer molecular specificity to analytes, and QD can be used as active or passive sensors in high-resolution cellular imaging [9, 10].

3 Nanocomposites for Biosensing

a. Basics of nanocomposites and their properties

Nanocomposites (NCs) are a subset of hybrid materials that are created by combining two or more nano-sized entities under specific experimental conditions. These materials play a significant role in various scientific and technological fields. By optimizing the combination of matrix and filler, the properties of nanocomposites can be adjusted. For instance, conducting polymers (CPs) can act as the matrix and nanomaterials as the filler, resulting in hybrid inorganic–organic CP nanocomposites (CPNs) that possess excellent properties, making them ideal for electrical and biosensing platforms [11]. The properties of nanocomposites can be tailored by optimizing the experimental conditions and carefully selecting the matrix and filler materials. For instance, the addition of nanoparticles to a polymer matrix can improve its mechanical, thermal, and electrical properties. Similarly, the addition of carbon nanotubes to a metal matrix can enhance its strength, stiffness, and electrical conductivity. The tunability of nanocomposite properties makes them attractive for a wide range of applications, including electronics, aerospace, biomedical devices, energy storage, and biosensors [12].

Electrical sensing methods such as cyclic voltammetry, amperometry, potentiometry, capacitors, chemoresistance, and field effect transistors are well-suited for rapid detection of alterations in current, voltage, and resistance. Nanocomposites are superior to their counterparts due to their increased electrical breakdown strength during fabrication, higher melting temperature, magnetization, and charge capacity. Other benefits include modified electrical conductivity, corrosion resistance, dielectric, and

semiconductor behavior. These properties make NCs ideal for use in solar cells, supercapacitors, biosensors, electrochemical sensors, and more [13, 14].

While the matrix phase is not directly used for assembling electrical sensor arrays due to limited linear range of response and interference by ionic and redox species, a solid contact phase—ion to electron transducer can be constructed, where the matrix acts as solid support and nanomaterial acts as an ion to electron transducer. Recent advancements in NCs-based electrical sensors have improved their limit of detection (LOD), and they are being used in practical applications with statistical analysis methods [15]. NCs are also useful in biosensing platforms such as tissue engineering, bio-electrodes, drug delivery, and biosensors to detect biological and synthetic molecules due to their nanoscale size and shape, biocompatibility, and biodegradability. Nanomaterials-based sensor arrays are in high demand for electrochemical sensors, biosensors, gas sensors, and immunosensors due to their exceptional performance in energy conversion performance, emission devices, integrated circuit-based nano-devices, medical devices, and more [16, 17].

Carbon nanomaterials, metal nanoparticles, and metal oxide nanoparticles can be embedded into conducting polymeric matrices to form CPNs with improved electrical, magnetic, thermoelectric, and biosensing properties. The incorporation of metal oxide nanoparticles into CPs has been extensively researched, with significant applications including supercapacitor electrodes, light-emitting diodes, transistors, electrochromic devices, electrochemical capacitors, photovoltaic cells, biosensors, gas sensors, actuators, and more [17].

The recent trends in nanocomposite synthesis by embedding carbon nanomaterials, metal and metal oxide nanoparticles into conducting polymers such as PANI, PPy, and PEDOT, and to examine their effect on the overall physical and chemical properties of CPNs. The article also highlights the utility of CPNs as biosensors, electrochemical sensors, gas sensors, therapeutic targeting sensors, and immunosensors [18].

b. **Types of nanocomposites used in biosensing**

QDs have special optical and electrical characteristics that make them valuable for a variety of applications, including biosensing. QDs are frequently used in nanocomposites along with other elements to enhance their functionality and compatibility with biological systems. As compared to QD, their nanocomposites display several novel traits and qualities as a result of the quantum confinement effect and edge effect.

1. Graphene-based QD

With a size range of 1–50 nm, graphene particles are stacked in a honeycomb shape to form GQD, a unique class of zero-dimensional semiconductor nanocrystals 19. GQD exhibits more advanced features compared to graphene due to the substantial change in physical size, including larger specific surface areas, more surface-active sites, and more accessible edges. Additionally, the functional groups included in GQD enable them to achieve superior solubility as well as stronger functionalization

with organic, inorganic, or biological moieties for the development of electrochemical biosensors [20, 21]. Also, GQDs have a high photoluminescence quantum yield, which means that they emit strong fluorescent signals when excited by light. This property makes them useful for fluorescent imaging and detection applications. For example, Sun et al. developed an electrochemical immunosensor for the detection of heat shock protein 70(HSP70) by using polyaniline functionalized GQD. Under optimized conditions, the fabricated sensor exhibits a wide linear range of 0.0976-100ngmL^{-1} and LOD of 0.05ngmL^{-1} [22]. Potiphor's group fabricated an electrochemical biosensor based on gold nanoparticles/graphene quantum dots/graphene oxide film-modified three screen-printed electrodes (AuNPs/GQD/GO/SPCE) for the simultaneous detection of three different microRNAs (miRNA-21, miRNA-155, miRNA-210). Due to the synergistic effect of the modified electrode, the biosensor exhibits a wide linear range from 0.001pM-1000pM with LOD of 0.04fM, 0.33fm, and 0.28fM for miRNA-21, miRNA-155, miRNA-210 respectively [23].

2. Chalcogenide based QD

Chalcogenide QDs, which are the most important fluorescent probes in the VI group, are derived from metallic chalcogenides such as cadmium-selenium (CdSe), cadmium-titanium (CdTe), cadmium-sulfur (CdS), and zinc-sulfur (ZnS) 24. Due to their optical and electrochemical qualities, these QDs have attracted a lot of attention. With their superior water solubility, high stability, wide bandgap, and good fluorescence qualities, chalcogenide QDs have a variety of uses in in-vivo imaging, diagnostics, and optoelectronic devices with a 1–20 nm wavelength range [25]. Due to these outstanding properties, Chalcogenide-based QD are prospective candidates for future sensor devices. For example, Moazampour et al. used ZnS functionalized L-cysteine to design an electrochemical genosensor to detect miR-200a, an ovarian cancer biomarker. Under optimized conditions, the dynamic linear range and LOD of miR-200a were found to be 1.0fM–1.0μM and 8.4fM respectively [26].

3. MXene-based QD

MXenes are a family of two-dimensional (2D) transition metal carbides, nitrides, and carbonitrides with unique electronic, mechanical, and chemical properties. Regarding their 2D-QD, they exhibit high surface area, high biocompatibility, and excellent photoluminescence properties which makes them suitable for biosensing applications. For example, Jiang et al. and his colleagues reported a nitrogen-doped Ti3C2 MXene QD (N-Ti3C2 QD) based electro-chemiluminescent immunosensor for the detection of mucin 1(MUC1). In this work, N-Ti3C2 QD shows ECL emission peaks at around 612nm and higher quantum efficiency than Ti3C2 QD which can serve as an effective ECL emitter to fabricate ECL immunosensor [27]. Recently, a glucose electrochemical biosensor was constructed for the detection of glucose using PEDOT: PSS/Ti3C2/GQD modified SPCE. The synergistic effect and excellent electrical conductivity of the modified electrode enhance the electrochemical performance of the enzymatic biosensor, achieving a LOD of 65μM [28].

4. Doped QD

Doping technology has steadily been used to create unique QD with good functionalities and extraordinary features in order to enhance the photoluminescence capabilities of QD. In biosensing applications, doped QD can be used as fluorescent. Labels to detect and quantify biological analytes. For example, In 2020, Zang et al. and his colleagues synthesized boron-doped carbon QD(B-CQD) for the detection of amoxicillin antibiotics via fluorometry. The fabricated sensor exhibits a superior linear range and high stability with an LOD of 0.825μM [29].

4 Quantum Dots-Based Nanocomposites for Biosensing

Nanomaterials are conventional materials that have at least one dimension on the order of nanometres, while nanocomposites are combinations of two or more types of nanomaterials that result in a new integrated material system, still in the nanorange. Nanoparticles are a structural term of nanomaterials, describing particles with a size smaller than 100 nm, while QDs are nanoparticles with a size of less than 10 nm, and are characterized by the dominant quantum confinement effect [14]. These distinctions will be important to keep in mind when discussing the unique properties and potential applications of these materials in various fields.

Nanomaterials for biosensing possess distinct characteristics in electronics, optics, mechanics, and thermal properties, making them an emerging material for developing new analytical methods for many analyses. Nanoparticles like quantum dots, nanowires, nanotubes, nanorods, and nanofilms have attracted attention in recent years. These materials have excellent optical and electrical properties closely linked to their sizes [30]. Quantum dots, for instance, are nanoscale semiconductor materials, such as cadmium selenide (CdSe), InAs, CdS, GaN, InGeAS, CdTe, PbS, PbSe, and ZnS. The controllable size parameter combined with the effect of quantum restriction makes QD exhibit extraordinary optical and electrical properties 12. Consequently, QDs have become labeling materials for biosensors, optical probes for imaging, and biological detection. They offer high sensitivity and selectivity in detecting specific analytes and can significantly increase the density of electrochemical signals in detection systems [31]. QD-based sensors have proven to be highly suitable for developing highly accurate, rapid, and selective analytical instruments for detecting particular analytes [32].

Researchers are increasingly interested in the fields of electrochemistry and nanomaterials, as nanomaterials are considered ideal for creating sensors. QD has been used successfully for developing biosensors. We include the selected methods in Table 1 for identifying various targets with various quantum dots.QDs, in particular, exhibit extraordinary optical and electrical properties due to their controllable size parameter and the effect of quantum restriction [33]. Their luminescence color varies with alterations in their size, making them well-suited as fluorescent probes

in medical diagnosis and imaging. However, the use of heavy metals such as CdSe, CdTe, and CdS in QD can lead to degradation and ion release, which is toxic under physiological conditions [34]. In the context of electrochemical sensors, there are both advantages and disadvantages associated with the use of quantum dots. The advantages include easy immobilization of bioreceptors, which enhances the possibility of the electroanalysis of multiple analytes, easy functionalization, and attractive electrochemical properties. On the other hand, the disadvantages include low stability, easy aggregation, and difficulty in controlling particle size [3, 4].

a. Introduction to quantum dots-based nanocomposites

This chapter discusses the use of QD in biosensors and highlights the importance of choosing the correct passivating agent to maintain their electronic properties and optical properties for real-life applications. Surface ligands such as metal–organic frameworks (MOFs), molecularly imprinted polymer (MIP), aptamers, multiwalled carbon nanotubes (MWCNTs), graphene quantum dots, and carbon QD can be used

Table 1 Selected QD-based electrochemical studies for different active compounds

QD type	Analyte	Linear range	Biomolecule	Ref.
Ni-doped CdTe	Pyrazinamide	2–100 μM	Plasma samples	[36]
CdTe	Dopamine	0.5–10 μM	Biological fluids	[68]
CdTe	Propafenone	0.003–7.0 μg mL^{-1}	Human serum	[69]
MoS2/CdTe	Tetracycline	0.1–1 μM	Milk samples	[70]
CdTe–Con A	Lipopolysaccharide and Serratia marcescens	10–90 fg/mL	–	[71]
CdSe	Urea	1–120 mM		[72]
CdTe	Salbutamol	6.27 × 10 − 8 to 2.09 × 10 − 7 M	Pig urine samples	[73]
Pd-doped CdTe	Diazinon	2.3–100 μM	Environmental water samples	[74]
CdS@MOF	Carcinoembryonic antigen	–	Human serum samples	[75]
CdTeS @SiO2	Folic acid	5–80 μM	Serum samples	[76]
α-FeOOH@CdS/Ag	17β-estradiol	0.01–10 pg. mL^{-1}		[77]
ZnCdS QD@MIP	Ascorbic acid	1–500 μM	Vitamin C tablets	[78]
MoS2/GQD	Caffeic acid	0.38–100 μM	Red wine samples	[79]
CdTe	Double-strand DNA	0.0874 μg mL^{-1} and 20 μg mL^{-1}	Synthetic samples	[80]
Polymer CdTe/CdS	Glucose	0.2–5 mM	Human body fluids	[81]

to modify the surface of QD for various sensor applications [35]. The use of silica nanoparticles as encapsulating agents for QD, can increase the amount of QD on each target cell and improve the sensitivity and detection range of electrochemical immuno-biosensors. The use of transition metal oxides with variable valence states and doped transition metal ions such as Ni:CdTe is also explored as options for enhancing the sensitivity and selectivity of QD-based biosensors. Mesoporous silica nanoparticles are shown to provide higher loading sites and surface area for QD than solid silica [33].

QDs are being used as labeling materials for biosensors, which are devices that detect biological analytes such as proteins, DNA, and cells. QD are nanoscale particles that emit bright and stable fluorescent signals, making them an attractive component for optical probes used in cellular, tissue, or whole-body imaging and biological detection [33].

QD-based sensors offer high sensitivity and selectivity in detecting certain analytes in the chemical and biochemical sciences. QD has been integrated into electrochemical sensors to improve their selectivity and efficiency in detecting specific analytes. QDs can provide sharp and well-resolved voltammetry signals while also increasing the electrochemical signal density in the electrochemical detection system [36].

Sensors utilizing QDs are well suited for the development of highly selective, fast, and accurate tools for the detection of specific analytes in sensor technology. A review of the literature shows that QD-based sensors have been used to develop highly sensitive and specific biosensors for a wide range of applications, including medical diagnostics, environmental monitoring, and food safety. Figure 1 shows statistics on the number of articles published each year relating to nanocomposites based on QD for electrochemical sensing.

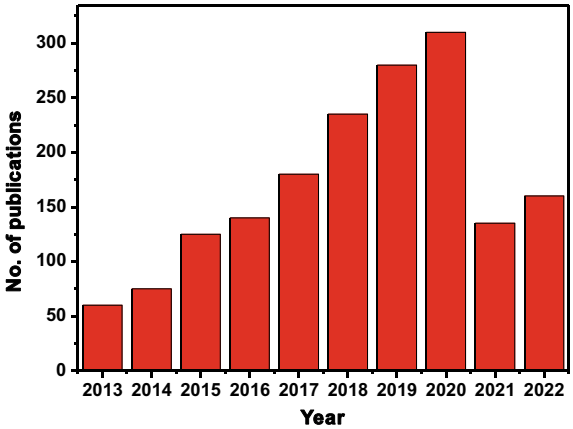

Fig. 1 Statistics of the number of publications per year related to quantum dot-based nanocomposites for electrochemical sensing

b. **Types of quantum dots-based nanocomposites for biosensing**

The popularity of QD as fluorescent biosensors is on the rise due to their robust photoluminescence, photostability, and sensitivity to changes in oxidative and acidic conditions. Additionally, QD can be modified to target specific biomolecules, allowing for the detection and measurement of various biomolecules in complex biological samples [37–39]. Moreover, QD has been integrated with other elements to boost their biosensing functionalities.

1. **QD-Nanocomposite Biosensors Based on Föster**

Föster resonance energy transfer (FRET) is a phenomenon where energy is transferred between a donor fluorophore and an acceptor fluorophore when they are in close proximity to each other. QDs are an ideal fluorescent donor due to their strong fluorescence, tunable narrow emission, and broad excitation wavelength range [40]. In a FRET system, when the donor emission matches the acceptor absorbance, energy is transferred from the excited donor to the acceptor, resulting in fluorescence emission of the acceptor fluorophore. FRET has been utilized in detecting dopamine by using a QD-dendrimer-AlexaFluor 488 labeled antibody, where the antigen–antibody reaction alters the distance between the donor and acceptor, leading to enhanced fluorescence emission [41]. DNA hybridization detection using molecular beacons is another well-known application of FRET. A nanocomposite consisting of QD-single-strand DNA and gold nanoparticles has been employed as a turn-on fluorescent sensor that activates to quantitatively measure target DNA. Quenchers such as carbon nanotubes, graphene, and graphene oxide have also been employed to fabricate nanomaterial-based biosensors. Dual-color sensors have been developed using QD-ruthenium complex nanocomposites for detection of double-strand DNA. The efficiency of FRET is influenced by two key factors which are the choice of the donor–acceptor pair and the distance between the donor and acceptor. Precisely designing the linkage materials is crucial to achieving the highest level of FRET efficiency in QD-nanocomposite biosensors [42].

2. **QD-Nanocomposite Biosensors Based on Electrochemiluminescence**

Electrochemiluminescence (ECL) has emerged as a promising technique for biosensing due to its ability to eliminate interference from excitation light sources in photoluminescence-based detections [43]. In ECL-based biosensing, QDs are electrically excited in media containing supporting electrolytes and ECL occurs when the oxidized or reduced QDs generated at certain electrochemical potentials interact with co-reactants like H_2O_2, $K_2S_2O_8$, and O_2 [44].

To enhance the sensitivity and specificity of ECL-based biosensors, QDs have been integrated with different nanomaterials such as multi-walled carbon nanotubes (CNTs), gold nanoparticles, poly(diallyldimethylammonium chloride) (PDDA), and graphene oxide to facilitate the electron injection process. For example, the combination of QD and CNTs has been shown to deliver a five-fold increase in ECL intensity and a positive shift of ECL starting voltage [45, 46]. Similarly, the use of CNT-QD-PDDA-gold nanoparticle composite films has been demonstrated as an immunosensor [47].

Interestingly, gold nanoparticles have been shown to both enhance and quench QD ECL through fluorescence resonance energy transfer (FRET), depending on the structure of the nanocomposite and the distance between QD and the gold nanoparticle. Gold nanoparticles can act as quenchers to eliminate QD fluorescence via FRET, but can also enhance ECL intensity when they are linked with QD through a hairpin-structured DNA molecule at close proximity [47].

Until now, the majority of the ECL biosensors utilizing QDs have been designed to identify biomolecules or bioreactions involving co-reactants actively involved in the ECL process. However, a universal ECL biosensor for the detection of various biomarkers using specially tailored QD nanocomposites still needs to be investigated.

3. QD-Nanocomposite Biosensors Based on Electrochemistry

The use of QD in nanocomposites for electrochemical sensors and photoelectrochemical solar cells has been demonstrated to have great potential for improving detection sensitivity and specificity. QDs are employed in electrochemical sensors as large-surface-area carriers to enhance enzyme loading and improve substrate detection in enzymatic reactions. Nanostructured composites incorporating QDs can also facilitate direct electron transfer between the redox centers of enzymes and the electrode, promoting direct electrochemistry, resulting in biosensors with good sensitivity and specificity [48, 49].

In photoelectrochemical solar cells, QDs are hybridized with other materials such as CNT, titanium dioxide (TiO2), and graphene to produce QD-based photoelectrochemical solar cells. When light irradiates the QD, it induces the dissociation of electrons and holes in the conduction and valence bands of QD, respectively. Based on the principle that electrons are injected into an electrode to produce a photocurrent when band energy is favorable and that holes are scavenged by suitable electrolytes to regenerate QD, QD-based photoelectrochemical sensors can be fabricated by measuring the change of photocurrent. This label-free approach is suitable for the detection of any antibody-antigen pair [50, 51].

As nanotechnology research continues to advance, the development and utilization of more energy-efficient and biocompatible QD nanocomposite are expected and used to enhance the sensitivity and specificity of diagnostic detection [49].

c. Functionalization of quantum dots-based nanocomposites for biosensing applications

QDs are semiconductor nanocrystals with unique optical and electronic properties, making them attractive for use in biosensing applications. However, to use QD for biosensing, they need to be functionalized with biomolecules such as antibodies, aptamers, or DNA strands that can specifically recognize and bind to target molecules.

Various methods, including covalent or non-covalent bonding, can be used to functionalize QD. Covalent bonding is the process by which biomolecules attach to the surface of QD by chemical reactions such as the formation of amide or thiol-ene bonds. On the other hand, non-covalent bonding involves the electrostatic interaction between positively charged QD and negatively charged biomolecules. The streptavidin–biotin system is a popular method for bioconjugation and one of the strongest

noncovalent biological protein–ligand interactions known to date. For example, Rahul et al. synthesized poly-L-Lysine (PLL) functionalized graphene QD(GQD) for the detection of carcinoembryonic antigens (CEA). Prepared PLL-GQD showed improved fluorescence lifespan, high quantum yield, outstanding photostability, biocompatibility, and higher cellular uptake as compared to nonfunctionalized GQD [52]. Sopan et al. fabricate a biosensor for the detection of para-aminohippuric acid using poly-L-glutamic acid functionalized graphene QD(PLGA-fGQD) embedded on UiO-66-NH2 MOFs). In this work, GQD was synthesized from bovine serum albumin by using the hydrothermal method. The surface modification of GQD with PLGA not only enhances the optical properties of GQD but also results in high quantum yield and responsiveness [53]. Rahul et al. developed a fluorescent-based lung cancer biomarker using polyethyleneimine functionalized graphene quantum dots, enhancing the stability and fluorescence lifetime [54]. Aerzoo et al. synthesized a hybrid ink using silver nanoparticles and D-penicillamine functionalized graphene QD for the detection of CA 125 biomarkers in human plasma samples. The developed immunosensor exhibits a good performance with a linear range of 0.001-400U ml-1 and a limit of quantification of 0.001U mL^{-1} [55]. Mayank et al. fabricated a fluorescence-based immunosensor for ferritin detection using amine-functionalized graphene quantum dots. Under optimized conditions, the engineered fluorescent immunosensor shows a linear range of 10-4000ng mL^{-1} and LOD of 0.723ng mL^{-1} [56]. Chen et al. constructed a label-free surface plasmon resonance (SPR) aptasensor for the detection of N-gene SARS-CoV-2 by using niobium carbide MXene QD which results in high bio affinity towards aptamer and enhances SPR signal [57]. Yao et al. used sulfhydryl-functionalized CQD as a fluorescent probe to detect heavy metal ion Hg2 + in lake water and wastewater samples. Due to the production of nonfluorescent ground-state complexes and the electron transfer interaction between HS-CQD and Hg2+ , the resulting HS-CQD were able to detect mercury ions Hg2+ quickly and sensitively through fluorescence quenching. The fabricated sensor obtained a linear range from 0.45μM–2.1μM with LOD of 12nM [58].

In addition to biomolecule functionalization, QD can be incorporated into various nanocomposites for biosensing applications. For example, QD can be encapsulated within silica nanoparticles to improve their stability and biocompatibility. QD can also be incorporated into hydrogels, polymers, or liposomes to create nanocomposites with unique properties.

Overall, the functionalization of QD-based nanocomposites offers promising opportunities for the development of biosensors with high sensitivity and specificity, making them valuable tools for various applications.

d. **Applications of quantum dots-based nanocomposites in biosensing**

1. **In Pharmaceutical industry**

In 2018, Tang et al. developed an electrochemical sensor that used a glassy carbon electrode (GCE) with graphene quantum dots (GQDs) to measure hydroquinone (HQ) and catechol (CC). The electrochemical impedance spectra were used to

describe this sensor's design through electrodeposition method. In the electrochemical measurement, the proposed GQD sensor showed excellent sensitivity, repeatability, and reliability with a detection limit of 0.08 μM in the range of 0.5 μM to 100 μM. In river water samples, simultaneous HQ and CC detection using a GQD/GC electrode was carried out with satisfactory recovery. In this study, the superior electrocatalytic and conductivity capabilities of the proposed sensor as well as its high precision, dependability, and repeatability in electrochemical measurement were used for HQ and CC [59].

Pasandideh-Nadamani and colleagues created a unique, incredibly sensitive, and extremely selective carbon paste electrode (CPE) in 2016 (33). Through the use of X-ray diffraction (XRD) and transmission electron microscopy (TEM) techniques, they were able to create CdS QD that were remarkably stable. Using an in-situ method and a thiosulfate precursor, CdS QD were produced. Without using any separation steps in the mixture, the electrochemical determination of p-aminophenol (PAP) and acetaminophen (Ac) was studied [60].

Algarra and colleagues have developed a modified glassy carbon electrode (GCE) with carbon QD(CQD) for the electrochemical measurement of dopamine and uric acid (34). The Hummers method was used to create CQD from graphite, and they were then examined using tools like the TEM microscope, XPS, Raman, solid-state NMR, and FTIR-ATR spectroscopies. The electrochemical analysis of both compounds revealed that the peak current in the CQD-GCE was significantly increased as compared to the bare glassy carbon electrode. The suggested sensor demonstrated good sensitivity using Linear Sweep Voltammetry (LSV). The lower limits of detection for uric acid and dopamine were determined to be 1.3 and 2.7 μM, respectively [60].

2. **In antibiotic detection**

Using magnetic nanoparticles (MNPs) and QD, Chen et al. created a suitable magnetic-QD (MNPs-SiO2-QD) material for the qualitative and quantitative detection of four antibiotics with a high percent recovery [61]. Meng et al. developed a novel technique that used fluorescent CdTe QD as a backdrop material to assess five quinolone antibiotic residues in food [62]. Li et al. developed a tetracycline (TC) detection method using GQD and Eu3+, and this system demonstrated outstanding potential for the detection of TCs in environmental and biological materials [63]. To identify tetracycline (TC) in serum samples, Zhang et al. created a novel fluorescent probe employing the hybrid QD/mesoporous silica/MIP (QD/MS/MIP) [64]. In order to identify enrofloxacin (ENR) in chicken muscle tissue, Chen et al. developed a competitive fluorescence-linked immunosorbent assay (c FLISA), which is appropriate for detecting veterinary medication residues [65]. To detect antibiotics, many QD types have been created and functionalized.

3. **QD and Nanocomposites in Cancer Detection and Therapy**

The use of molecular imaging with QD linked to biorecognition molecules is expected to play a significant role in cancer diagnosis, especially for the early detection of cancer lesions. QD can be used for cell tracking to study cell division and metastasis

and can act as unique markers for tracking cancer cells during metastasis. Whole-body NIR optical imaging is a powerful technique that allows the observation of complex biological phenomena with minimum invasiveness [66]. QD emitting in the NIR region can be used as markers in sentinel lymph node mapping, a major procedure in cancer surgery, whereby the lymph node closest to the organ affected is monitored for the presence of roaming cancer cells. QD can also be conjugated with biomolecules such as peptides and antibodies to target tumors in vivo. Conjugation of QD with specific ligands enables the detection of cancer-related proteins and aids in diagnosis and imaging of breast cancer. The future of QD in cancer diagnosis and treatment lies in identifying metastatic cancer cells, quantitatively measuring the level of specific molecular targets, and providing biodynamic markers for target inhibition [67].

4. **Various biosensing application**

Pourghobadi et al. created TGA-capped CdTe QD to allow for the visual assessment of dopamine traces. As a result of the interaction between TGA-CdTe QD and dopamine, the fluorescence intensity of the samples was examined. A rise in dopamine concentration causes a sharp decline in fluorescence intensity. We created a simple, fluorescence-sensitive approach for the measurement of dopamine taking into account this tendency [68].

Hydrophilic QD can be employed as molecular probes to find minute levels of propafenone, claim Wang et al. Propafenone was able to mix with CdTe QD that had been treated with thioglycolic acid in the weak acid through electrostatic attraction and hydrogen bonding. The interaction of CdTe QD with propafenone has been investigated using spectral analysis, spectra, RRS, UV–vis absorption, and fluorescence. Propafenone concentrations of ppb (ng mL^{-1}) in serum samples can be found in less than 30 min using the RRS method [69].

Using Liang et al.'s CdTe and MoS2 QD, tetracycline was found in milk. First, under 365 nm excitation, MoS2 QD emits blue light at 433 nm, while CdTe QD emits yellow light at 573 nm. We created a fluorescent sensor with dual signals at 433 and 573 nm using MoS2 QD and CdTe QD. As tetracycline concentration is raised, MoS2/CdTe-based sensors exhibit a decrease in fluorescence intensity, and 573 nm is quenched more visibly than 433 nm [70].

As a successful new biosensor for detecting LPS at concentrations of fg/mL, TGA-capped conjugated CdTe QD was created. In the high-resolution transmission electron image of the CdTe QD, high crystalline lattice planes with an average size of 4–5 nm were visible. The adsorption of Con A onto CdTe QD surfaces was caused by the electrostatic attraction force between positively charged Con A species and negatively charged CdTe QD surfaces [70].

The fluorescent carbon nanoparticles known as carbon QD (CDs), which are less than 10 nm in size, have good biocompatibility, water solubility, and distinctive optoelectrical characteristics 15. Bioimaging, biochemical and chemical analyses, photocatalysis, and white light-emitting devices have all been effectively employed with them. The ECL behavior of water-soluble CDs has recently been described, and Zheng et al. have suggested the underlying mechanism. The usage of CDs as an ECL

reagent may now have a hopeful future thanks to this research. Here, we explore the PL and ECL behaviors of Ru(bpy)32 + and CDs in solution for the first time, and we create CDs as nanoprobes for identifying Ru(bpy)32 + species 61.

Recent advances in quantum dots-based nanocomposites as biosensors

There have been several recent biosensing applications using QD-based nanocomposites. Here are some examples:

1. **Detection of cancer biomarkers**

QD-based nanocomposites have shown great potential in cancer biomarker detection due to their unique optical and electronic properties. To create QD-based nanocomposites for cancer biomarker detection, QDs are typically functionalized with a biomolecule such as an antibody or aptamer that specifically binds to the biomarker of interest. For example, Mehrnaz et al. developed an immunosensor for the detection of prostate cancer biomarkers in human serum using AuNP@MWCNTs-GQD. The surface of a glassy carbon electrode was coated with AuNPs@MWCNTs-GQD to act as a signal amplifier, and then an antibody against the prostate-specific antigen (PSA) was immobilized on the modified electrode. Under optimized conditions, the fabricated immunosensor determined the PSA concentration in a linear range of 1–10,000 $pgmL^{-1}$ and LOD of 0.48 $pgmL^{-1}$ [82]. Neeraj et al. synthesized GQD by electrochemically exfoliating waste dry batteries and fabricated an electrochemical sensor to detect CD44 cancer biomarkers. The fabricated biosensor shows high sensitivity with a LOD of 2.11fg/ml83. Qin et al. developed an electrochemiluminescence immunosensor for the detection of CA15-3, a breast cancer biosensor using AuNPs/CQD-PEI-GO composite. The developed immunosensor has high selectivity and reproducibility for the detection of CA15-384. Koushik et al. used single-walled carbon nanohorns and nitrogen-doped GQD nanocomposite for the detection of α-fetoprotein, a cancer biomarker. In this work, GCE was modified by N-GQD@SWCNHs and then anti-α-fetoprotein was immobilized. The incorporation of N-QD and SWCNHs enhances the conductivity and sensitivity of the fabricated immunosensor with a LOD of 0.25 $pgmL^{-1}$ 85 Overall, QD-based nanocomposites show great promise in cancer biomarker detection and could have significant impacts on early diagnosis and treatment of cancer.

2. **Detection of foodborne pathogens**

Foodborne infections continue to pose a serious threat to human health worldwide and cause significant economic losses. Foodborne pathogens come in a variety of forms, including bacteria, fungi, viruses, and parasites [86]. Additionally, E.coli, Salmonella enterica, Listeria monocytogenes, Campylobacter jejuni, Staphylococcus aureus, and Clostridium perfringens are the most prevalent foodborne pathogens. QD-based nanocomposites have shown promising results in detecting foodborne pathogens due to their unique optical and electronic properties. One advantage of using quantum dot-based nanocomposites for pathogen detection is their sensitivity as they can detect low levels of pathogens, even down to a single cell, which is important for ensuring the safety of food products. Additionally, they can provide

rapid detection, which is crucial for preventing the spread of foodborne illness. For example, Wang et al. fabricates an electrochemical immunosensor for the detection of Staphylococcus aureus using Ag2S QD loaded with dendritic mesoporous silica nanospheres. With a detection limit of 2CFUml^{-1} and a wide linear range from 10CFUmL^{-1} to 108CFUmL^{-1}, the fabricated immune biosensor shows high selectivity and stability in the electrochemical measurement [87]. In order to create the ECL system for smartphones, Li et al. used GQD/Ag nanoparticles (GQD/AgNPs). They successfully completed the real-time detection of E. coli, which could measure E. coli in the range of 10–107 CFUmL^{-1} 88. With a low detection limit of 0.05 CFU/mL, GQD doped with nitrogen was combined with non-metallic, two-dimensional, hydrated defective tungsten oxide to create a photoelectrochemical aptamer biosensor for E. coli detection. The fabricated biosensor provides new opportunities to increase the efficiency of both disease prevention and diagnosis [89].

3. **Environmental monitoring**

QD are semiconductor nanoparticles that have unique optical and electronic properties, such as size-dependent fluorescence emission, high photostability, and tunable surface chemistry. These properties make QD ideal for environmental monitoring applications, as they can be used as sensors for detecting pollutants, heavy metals, pesticides, antibiotics, and other environmental contaminants. For example, Korram et al. develop a fluorescent sensor using cadmium telluride QD (CdTe QD) for the detection of various organophosphate pesticides including paraoxon, dichlorvos, malathion, and triazophos in apple and tomato juice [90]. By employing palladium ions as a dopant during the hydrothermal process, Najafi et al. reported Pd: CdTe QD in the presence of diazinon, and fluorescence intensity linearly decreases. Diazinon was discovered in environmental water using this unique nanoprobe at extremely high sensitivity and selectivity. Additionally, Pd: CdTe QD's excellent cytotoxicity assay demonstrates their enormous potential in biotechnology and medicine [91]. Roushani et al. [92] fabricate an aptasensor using AgNPs/GQD-N-S/AuNPs nanocomposite for the detection of streptomycin. The fabricated biosensor functions at a low concentration level of pgmL^{-1} [92] Muthusankar et al. used N-CQD@Co_3O_4 /MWCNT blend nanocomposite as an electrode modifier for the simultaneous detection of anticancer drug flutamide and antibiotic nitrofurantoin. The fabricated electrochemical show a wide linear range of 0.05–590 μM and 0.05–1220 μM and LOD values were 0.0169 μM and 0.044 μM for flutamide and nitrofurantoin respectively [93]. Li et al. created an N-doped-CQD-GO modified GCE (NCQD-GO/GCE) sensor for the detection of Cd(II) and Pb(II). The fabricated sensor shows linear detection ranges of 100.357 nM–100.357 μM and 100 nM–50 μM for Cd(II) and Pb(II) respectively and corresponding LODs of 66.518 nm and 5.647 μM [94]. Pizarro et al. modified a GCE using GQD and nafion to create an eco-friendly sensor that can determine the concentrations of Cd(II) and Pb(II) in seafood [95].

Overall, these examples highlight the versatility and potential of QD-based nanocomposites for biosensing applications in various fields, including healthcare, food safety, and environmental monitoring.

Potential Future Developments in Quantum Dots-Based Nanocomposites for Biosensing

QD are nanoscale semiconductor particles that exhibit special optical and electrical characteristics, such as size-tunable fluorescence and high quantum yield. These characteristics have made QD a popular choice for biosensing applications. QD-based nanocomposites have been utilized in biosensing to detect a range of biomolecules with high sensitivity and specificity, including proteins, nucleic acids, and small molecules. Future advancements in QD-based nanocomposites for biosensing could include the following:

1. **Multiplexed detection**: Multiplexed detection involves the simultaneous detection of multiple analytes in a single sample. QD-based nanocomposites have the potential to be used for multiplexed biosensing, as they can be functionalized with different recognition molecules that bind specifically to different target analytes. Multiplexed detection using QD-based nanocomposites could enable the rapid and accurate diagnosis of complex diseases.
2. **High-throughput screening**: QD-based nanocomposites could be used for high-throughput screening of drugs and other compounds. By functionalizing QD with specific receptors or enzymes, it is possible to develop biosensors that can detect the activity of these molecules in a high-throughput manner.
3. **Imaging**: QD-based nanocomposites could be used for cellular and molecular imaging. QD can be conjugated with biomolecules, such as antibodies or peptides, to target specific cells or tissues. The fluorescence microscopy enables the visualization of specific cells or tissues.
4. **Portable biosensors**: QD-based nanocomposites have the potential to be used in portable biosensors for point-of-care testing. The small size of QD and their high sensitivity make them ideal for use in miniaturized biosensors that can be used in remote or resource-limited settings.
5. **Therapeutic applications**: QD-based nanocomposites could be used for therapeutic applications, such as targeted drug delivery. By functionalizing QD with specific targeting molecules, it is possible to deliver drugs specifically to the cells or tissues that need them, reducing side effects and improving efficacy.

Overall, QD-based nanocomposites hold great promise for the development of biosensors with improved sensitivity, specificity, and portability, as well as for therapeutic applications. Continued research in this area is likely to yield further exciting developments in the future.

5 Challenges and Future Prospects

(1) Challenges and limitations of quantum dots-based nanocomposites as biosensors

QD are nanocrystals that exhibit unique optical and electronic properties due to their size-dependent confinement effects. These properties make them attractive for use in biosensors, which are devices that, quantify biological analytes. However, there are several challenges and limitations associated with QD-based nanocomposites as biosensors.

1. **Biocompatibility**: One of the major challenges of using QD in biosensors is their potential toxicity to biological systems. Many QDs are made of heavy metals, such as cadmium, which can be toxic to cells. Therefore, it is important to ensure that QDs are coated with biocompatible materials to prevent them from interacting with biological systems in a harmful way.
2. **Stability**: QDs can be susceptible to degradation and aggregation, which can affect their optical properties and lead to inaccurate measurements. Therefore, it is important to ensure that QDs are stable in biological environments and that their optical properties are not affected by the presence of biological molecules.
3. **Sensitivity and selectivity**: QD-based biosensors must be highly sensitive and selective in order to detect low concentrations of biological analytes. However, QD can be prone to nonspecific binding, which can decrease their selectivity. Therefore, optimizing the surface chemistry of QD to enhance their selectivity and reduce nonspecific binding is important.
4. **Signal-to-noise-ratio**: QD-based biosensors must be able to detect signals in the presence of background noise, which can be challenging due to the small size of QD and the low concentration of analytes in biological samples.
5. **Cost**: QDs can be expensive to produce, which can limit their use in practical applications. Therefore, it is important to develop cost-effective methods for producing QD and incorporating them into biosensors.

In summary, while QD-based nanocomposites offer unique optical and electronic properties that make them attractive for biosensing applications, there are several challenges and limitations that must be addressed in order to ensure their effectiveness and safety in biological systems.

(2) Future Prospects and potential impact of quantum dots-based Nanocomposites on Biosensing

QD-based nanocomposites have the potential to revolutionize biosensing by enabling rapid and sensitive detection of biomolecules, pathogens, and other targets of interest. One of the key advantages of QD-based nanocomposites is their tunable emission spectrum, which allows for the detection of multiple targets simultaneously using a single probe. Additionally, QD exhibits high quantum yields and photostability, which enables long-term monitoring of biological processes and reduces the need for frequent sample preparation.

Another advantage of QD-based nanocomposites is their versatility in terms of surface functionalization. The surface of QD can be modified with a variety of biomolecules, such as antibodies, aptamers, and peptides, to selectively bind to target molecules. This enables the development of highly specific biosensors for a wide range of applications, including disease diagnosis, drug discovery, and environmental monitoring.

Furthermore, QD-based nanocomposites have the potential to enable real-time, in vivo monitoring of biological processes. QD can be incorporated into various biological systems, such as cells or tissues, and their unique optical properties can be used to monitor changes in the local environment, such as pH, temperature, and oxygen levels.

In conclusion, while there are still challenges to overcome, the development of QD-base biosensors holds great promise for a wide range of applications in healthcare, environmental monitoring, and beyond.

6 Conclusions

In conclusion, quantum dots-based nanocomposites have shown great potential as biosensors due to their unique optical and electronic properties. By combining QD with various materials such as metals, polymers, and biological molecules, it is possible to design highly sensitive and selective biosensors for the detection of various biomolecules, including proteins, DNA, and viruses. Moreover, the versatility of QD allows for the development of multiplexed biosensors capable of detecting multiple analytes simultaneously. The integration of QD into nanocomposites offers several advantages over conventional biosensors, including increased sensitivity, selectivity, and stability. Additionally, the use of nanocomposites allows for the development of biosensors with a wide range of optical and electronic properties, enabling the detection of biomolecules using a variety of sensing modalities. However, there are still challenges that need to be addressed to fully realize the potential of quantum dots-based nanocomposites as biosensors. These include issues related to toxicity, stability, and reproducibility. With continued research and development, these nanocomposites are likely to become increasingly important tools for the detection of biomolecules and the advancement of biomedical sciences.

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Quantum Dot-Based Nanocomposite as Metal Sensors

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Abstract The quest for searching new functional materials for efficient heavy metal sensors has motivated the development of productive hybrid materials in which the strengths and weaknesses of various materials are considered and the complimentary and synergetic functions of the individual materials are utilized for achieving better material performance. Quantum dots (QDs) are zero-dimensional nanomaterials with inherent optical and optoelectronic properties and thus are highly employed for heavy metal sensing. Recent advances in QD research have produced QDs-based nanocomposites by doping QDs with metals, conjugating QDs to molecules, polymers, magnetic materials, and biomolecules to improve hydrophilicity, and stability and enhance their sensing capabilities. This chapter describes the application of various QD-based nanocomposites as heavy metal sensors. The importance and involvement of materials such as metal-doped QD, QD-based polymeric, QD-based

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magnetic, QD-based metal–organic frameworks (MOFs), and QD-based bioconjugates are well described. The sensing mechanisms involved in the detection as well as challenges coupled with future projecions are also highlighted.

Keywords Quantum dots · Nanocomposites · Fluorescence · Metals · Sensing

List of Abbreviations

QD	Quantum dots
IFE	Inner filter effect
FRET	Fluorescence resonance energy transfer
PET	Photo-induced electron transfer
ACQ	Aggregation induced quenching
ACQ	Aggregation induced quenching
AIEE	Aggregation induced emission enhancement
AAS	Atomic absorption spectroscopy
ICP	Inductively coupled plasma
UV	Ultra-Violet
MOFs	Molecular organic frameworks
Er	Erbium
GQD	Graphene quantum dots
Eu	Europium
SET	Surface energy transfer
DET	Dexter energy transfer
PL	Photoluminescence
NIR	Near infrared
RBD	Rhodamine B derivative
PVDF-HFP	Poly (vinylidene fluoride-co-hexafluoropropylene)
TDDFT	Time-Dependent Density functional
LUMO	Least unoccupied molecular orbital
HOMO	Highest energy molecular orbital
CTSCD	Poly (β-cyclodextrin/chitosan
SQD	Sulphur quantum dot
MPA	Mercaptopropionic acid
ZAIS	Zn-doped $AgInS_2$
GSH	Glutathione
TEM	Transmission electron microscope
XPS	X-ray photoelectron spectroscopy
AES	Atomic emission spectroscopy
PSMA	Poly-(styrene-co-maleic anhydride
LOD	Limit of detection
CDs	Carbon dots
P-PEN	Phenolphthalin-grafted polyarylene ether nitrile

MNPs	Magnetic nanoparticles
NMs	Nanomaterials
EDC	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride
CMCN	Carboxymethyl chitosan
ZIF	Zeolitic imidazolate framework
DNA	Deoxyribonucleic acid
TSPP	Meso-tetra (4-sulfonatophenyl) porphine dihydrochloride

1 Introduction

The high demand for resources has increased industrial and municipal activities, which has increased the occurrence of heavy metal ions pollution. Heavy metal ions are important environmental pollutants that caught the attention of environmental scientists due to their water solubility, small ionic state dimension, and non-degradability [1, 2]. Although some heavy metals (Zn, Cu, and Fe) are essential for human health they can be toxic at elevated concentrations. Non-essential metals such as Cd, Pb, and Hg are toxic even at trace levels. The effects of their presence in aquatic water bodies include bioaccumulation in aquatic plants and animals and a tainted food chain. In human bodies, the existence of toxic heavy metal ions affects body organs such as the kidney, brain, reproductive system, and heart leading to various organ disorders [3]. In addition, the detection of intracellular metal ions is important to understand biological functions and various medical diagnoses [4, 5]. Thus, developing sensitive techniques for the detection and quantification of heavy metal ions in both water environments and biological matrices is crucial. Various techniques have been developed for monitoring levels of heavy metal ions including the popular spectroscopic (Atomic absorption spectroscopy (AAS) and inductively coupled plasma (ICP), and spectrophotometric systems (ultra-violet (UV)–Visible) [6, 7] and the emerging sensors based on functional materials such as fluorescent organic materials, nanomaterials, molecular organic frameworks (MOF) [8, 9].

The pursuit for fabricating novel functional materials capable of efficiently detecting heavy metal has expanded the synthesis of hybrid materials whose strengths and weaknesses are considered and the complimentary and synergetic functions of the individual materials are utilized for realizing enhanced material performance. Over the years, nano-based materials have emerged in the field of heavy metal ion detection. Among the nano-detection systems, quantum dot (QD)-based fluorescent sensors have gained popularity due to their low detection limits and fast response time offerings. Quantum dots are zero-dimensional nanomaterials with inherent optical and optoelectronic properties and thus are highly employed for fluorescence bio-imaging, photo-thermal disease treatments, and energy processes [8, 10]. Semiconductor QDs such as II-VI, I-III-VI type [8, 11–15], and carbon-based QDs such as carbon QDs and graphene oxide QDs [16, 17] have widely applied fluorescence detection of heavy metals in water. However, despite these unique properties, some

QDs aggregate in water due to their extreme hydrophobicity, and thus their applications are limited in aqueous environments. To circumvent this challenge, efforts have been made to convert them to hydrophilic materials through surface functionalization with hydrophilic functional groups such as acid or amine groups [14, 18]. Alternatively, recent advances have produced QD-based nanocomposites to improve hydrophilicity, stability and enhance their sensitivity to heavy metals. The frequently reported QD-based nanocomposites for heavy metal ion sensing include metal-doped QDs, QD-based polymeric, QDs-molecular organic frameworks (QDs-MOFs), QD-based magnetic, and QD-based bioconjugate nanocomposites. The significance of this chapter is to provide information on QD-based nanocomposites and their applications as metal sensors in aqueous and biological environments. First, the various metal sensing mechanisms involved are discussed. The challenges associated with QD-based nanocomposites in metal sensing, conclusions, and future outlooks are also fully elucidated.

2 Metal Sensing Mechanisms in QD-Based Nanocomposites

QDs are excellent fluorescent sensors because they show a notable change in their fluorescence signal as a result of a specific interaction with a given molecule (metal ions), to detect and correlate the changes to the analyte concentration [19]. The interaction between QDs and the analyte results in either a turn-off [20] or a turn-on of the fluorescence signal of the nanoparticle due to the recombination processes of the electron–hole pair [21]. In as much as standalone QDs efficiently detect the presence of metal ions in various media such as water and biological samples, their drawback is the loss of fluorescence due to agglomeration when exposed to ambient conditions, moreover, they contribute to secondary pollution of the medium hence the need for their stabilization and immobilization in other materials to form QD-based nanocomposites [22]. Literature has shown that even composites of QDs obey the same mechanism. The mechanisms with which the fluorescence turns-off or on are known as the fluorescence quenching and fluorescence enhancing mechanisms, respectively.

2.1 Fluorescence Quenching Mechanisms

Fluorescence quenching occurs when the interaction between the QDs (fluorophore) and the analyte (quencher) results in a reduction of the fluorescence peak intensity and hence reduced photoluminescence (PL) properties [23]. Understanding these mechanisms is vital to avoid possible interferences and make selective strategies [24]. The most reported quenching mechanisms in QDs are static quenching, dynamic quenching, fluorescence resonance energy transfer (FRET), photo-induced electron

transfer (PET), inner filter effect (IFE), aggregation-induced quenching (ACQ) and cation-exchange [25].

2.1.1 Static Quenching

Static quenching is a phenomenon that occurs when the interaction between the QD/fluorophore and the analyte/quencher forms a non-fluorescent, ground-state QD-quencher complex without emitting photons [26]. This mechanism could be linked to the formation of the ligand–metal complex [23]. Characteristics of static quenching are: (i) absorption spectra of the QDs become altered when the ground state complex is formed, (ii) an increase in temperature destabilizes the ground state complex and thereby reduces the efficiency of the static quenching, (iii) there is no significant difference between the QDs lifetime and that of ground state QDs-quencher complex [27].

2.1.2 Inner Filter Effect (IFE)

IFE is realized when the absorption spectrum of the metal ion overlaps with the emission spectra of QDs. This fluorescent decay mechanism is not a major quenching process because it does not involve radiative and non-radiative transitions in fluorescent measurements [23], but is caused by an attenuation of the excitation beam or absorption of the emitted radiation by an excess concentration of QDs or by the metal ion in the solution [28]. IFE cannot be categorized as a static or dynamic quenching process. The process can be confirmed when the PL intensity declines with the metal ion concentration but not the decay time. It has been reported to occur even when the distance between the QDs and the metal ion is more than 10 nm [29]. The process does not need energy transfer or chemical modification of the QDs [30].

Huynh et al. [31] produced erbium (Er)-doped graphene (G) QD nanocomposites via a one-pot hydrothermal method. The QD nanocomposite showed a good response to Fe^{3+} in the wide range of 0.01–140 μM with limit of detections (LODs) of 2.8 nM in PBS and 28 nM in human serum when excited at 360 nm and 730 nm for the down and up conversion respectively. The down conversion peak emerged at 440 nm and decreased proportionally with an increase in the concentration of Fe^{3+} (0–400 μm). The fluorescence lifetime decay of the Er-GQDs in the absence and the presence of Fe^{3+} was 7.4 ns signalling either a static or IFE quenching mechanism. However, due to the red-shift of the absorbance peak from 300 to 314 nm, the static mechanism was overruled because the red-shift suggests the formation of a new complex. Furthermore, the nanocomposite was found to be negatively charged while the metal ion was positive a phenomenon that allows electrostatic interaction between the nanocomposite and the Fe^{3+} resulting in static quenching of the fluorescence intensity. The excited electrons of hydroxyl groups on the surface of the Er-GQDs are transferred to the d orbitals of the Fe^{3+}.

Wang et al. [32] constructed a ratiometric fluorescence sensor using the fluorescence (467 and 616 nm) of nitrogen and cobalt (II) co-doped carbon dots (CDs) encapsulated in europium metal–organic frameworks (CDs@Eu-MOFs). The PL intensities of CDs@Eu-MOFs at 467 nm gradually declined with the increase of Cr(VI) concentrations from 0 to 500 μM, while the fluorescence intensity at 616 nm displayed insignificant changes. The observations implied that the adsorbed Cr(VI) blocks the excitation of composite and hence the fluorescence quenching at 467 nm, which indicated IFE quenching mechanism [33].

2.1.3 Dynamic Quenching Mechanism

During the dynamic quenching mechanism, the excited state of QDs return to the ground state by collision with the quencher via energy or charge transfer. The common examples of this type of mechanism include Forster resonance energy transfer (FRET), photon-induced electron transfer (PET) and rarely surface energy transfer (SET), dexter energy transfer (DET) mechanisms [26]. A simplified reaction that occurs during dynamic quenching is presented in Eq. 1.

$$F^* + Q \rightarrow F + Q \tag{1}$$

where * represents the excited state of QDs while Q denotes the quencher. This interaction obeys the Stern-Volmer relationship as indicated in Eq. 2 [24].

$$\frac{F_0}{F} = 1 + K_{SV}[Q] = \frac{\tau_0}{\tau} = 1 + kq\tau_0[Q] \tag{2}$$

where F and F_0 are the relative emission intensity of the donor (QDs) with and without the acceptor (quencher), respectively, K_{sv} is the Stern–Volmer quenching constant, [Q] is the concentration of the quencher, $\tau_{0,}$ and τ are the average lifetimes of the donor with and without the quencher, respectively, and k_q is the quenching rate constant.

The characteristics features of dynamic quenching are: (i) the fluorescence lifetime of the QDs changes in the presence or absence of the metal (ii) since this mechanism only affects the excited states of QDs the absorbance spectra do not display any changes (ii) raising the temperature increases the influence of dynamic quenching.

FRET

FRET occurs when non-radiative energy is transferred from excited QDs to a metal ion that is in the ground state through a dipole–dipole interaction [21, 34]. The application of QDs in FRET is easier when they function as donors because they can be excited at any wavelength as provided that excitation wavelength is shorter than the emission position. Also, QD emission can be tuned to overlap the absorbance spectra

of the quencher. It is quite challenging to use QDs as FRET acceptors because of their broad absorption which makes it hard to find a donor with a PL spectrum that overlaps with QDs but is not concurrently excited with the QDs [34]. To overcome the QD-FRET predicament that is experienced when QDs are acceptors, fluorescent lanthanides are often employed as donors [35]. Alternatively, the up conversion of nanoparticles (NPs) which are excited in the near-infrared (NIR) region enabling their excitation but not directly exciting QDs are used as donors [36]. The main indicators of the FRET mechanism include, (i) overlapping of the QDs PL spectrum with that of the quencher, (ii) reduction of the fluorescent lifetime of the QDs upon interaction with metal ions, (iii) the distance of the QDs and quencher should be in the range 10–100 Å [37]. Recently, a novel QDs nanocomposite fluorescent probe for Fe^{3+} detection in aqueous solution was constructed using three components, namely the CdTe QDs as the energy donor, Rhodamine B derivative (RBD) was the energy acceptor while gamma-cyclodextrin (γ-CD), functioned as the linker between the QDs and RDB. The CdTe@γ-CD@RBD nanohybrid exhibited a strong fluorescence emission at 600 under 290 nm excitation. The RBD did not show any absorbance in the absence of Fe^{3+} but there was an apparent peak at 559 nm when Fe^{3+} was added, indicating a strong interaction between RBD and Fe^{3+}. The absorption peak of RBD-Fe^{3+} at 559 nm overlapped with the PL peak of the QDs signalling a FRET mechanism. The consistent decrease of the fluorescent intensity of the nanohybrid probe with the increase in the Fe^{3+} concentration between 10 and 60 μM confirmed an adjustable FRET efficiency between the probe and the metal ion. Moreover, the nanocomposite recorded a fluorescence lifetime of 22.7 ns without the incorporation of Fe^{3+} which was significantly reduced to 4.4 ns after Fe^{3+} was added further demonstrating a FRET process from QDs to RBD-Fe^{3+} [38].

PET

PET is a reversible process that can either be reductive or oxidative. Reductive PET occurs when the QD accepts electrons from the quencher (metal ion) while oxidative PET is when the QDs donate electrons to the quencher [21]. So, in this quenching mechanism, QDs can function as donors or acceptors of electrons to form the cation and anion radicals respectively which is the ground state complex (QDs-acceptor). Oxidative PET is the commonly observed fluorescent response between QDs and transition metal ions [37]. The coordination complexes are formed between the QDs' surface functional groups and the metal ions which have unfilled orbitals. The transfer of electrons from the excited QDs to the unfilled orbitals of the metal ion leads to fluorescence quenching [42]. The PET quenching mechanism is confirmed when there is (i) a reduction of fluorescent life time of the QD composite-quencher (metal ion) complex which indicates that the process is dynamic, (ii) the kq of QD composite-metal ion is larger than 10^7 M^{-1} s^{-1} which is attributed either to electron or to energy transfer process, (iii) an overlap between the PL spectrum of QDs/QDs composite and the absorption band of the metal ion is less pronounced, (iv) the PL intensity decreases linearly with the increase of the metal ion while the absorption spectra remains

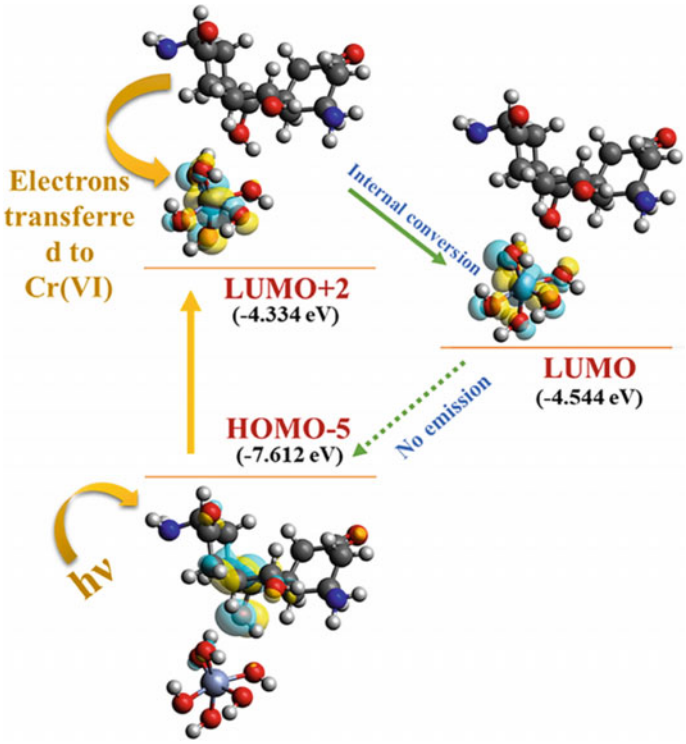


Fig. 1 Depiction of PET-based fluorescence quenching mechanism as per the TDDFT. Reproduced with permission from reference [22]

unchanged and finally (v) the PL intensity is restored with the addition of a chelating agent [39]. To enhance the stability and prevent the leaching of CQDs, Roy et al. [22] fabricated boehmite-carbon dot nanocomposite (BH@CQD) that was further encapsulated with a matrix of poly (vinylidene fluoride-co-hexafluoropropylene) (PVDF-HFP) polymer. The obtained nanocomposite served as a fluorometric sensor device to detect hexavalent chromium (Cr^{6+}) in water. A theoretical tool, the Time-Dependent Density functional (TDDFT) study showed that the quenching mechanism involved in the quenching detection of Cr^{6+} is PET. According to the TDDFT, upon excitation, the molecular orbitals at the Highest Energy Molecular Orbital (HOMO) −5 state go to the Least Unoccupied Molecular Orbital (LUMO) +2 state when QD composite-Cr^{6+} after a non-radiative internal conversion jump to the LUMO state of the complex system. During these transitions the molecular orbital shifts to the Cr^{6+} moiety and depicts electron transfer between QDs in the composite and the metal ion. Lastly, the PL quenching occurs because of a radiative transition that takes place from LUMO to HOMO-5 due to the orbital shifting towards the Cr^{6+} moiety and hence PET (Fig. 1) [40]. In simpler terms, excess Cr^{6+} ions block the fluorescence path of the QDs causing quenching [41].

2.1.4 Aggregation-Induced Quenching (ACQ)

ACQ is observed when the QDs display strong fluorescence in a dilute solution but the PL properties weaken and disappear at higher concentrations of the QDs [42]. The ACQ could result in (i) the loss of energy between the QDs, and the metal ion when they collide (ii) the generation of a non-radiated complex; and (iii) self-extinction due to excessive concentrations of the QDs [43]. For instance, Wang et al. [44] synthesized a very sensitive poly (β-cyclodextrin/chitosan (CTSCD)-sulphur QDs (SQDs) (SQDs/CTSD) nanocomposite via the self-assembly method for the selective detection of Ag^+ (Fig. 2). The SQD/CTSCD sensor detected Ag^+ in a good linear relationship in the range of 1.0×10^{-5} to 5.5×10^{-5} mol/L ($R^2 = 0.9992$) and a detection limit of ~85 nM. The negatively charged SQDs on the CTSCD chain coordinated with the positively charged silver ions to form S– Ag^+ –S bonds. The formed bonds are associated with non-covalent bonds resulting in Ag^+-SQD/CTSCD nanocomposite aggregates that lead to ACQ [44].

2.2 *Fluorescent Enhancement Mechanisms*

Fluorescence enhancement refers to the improvement or increment of the PL intensity when QDs interact with a molecule [23]. Fluorescent enhancement mechanisms can

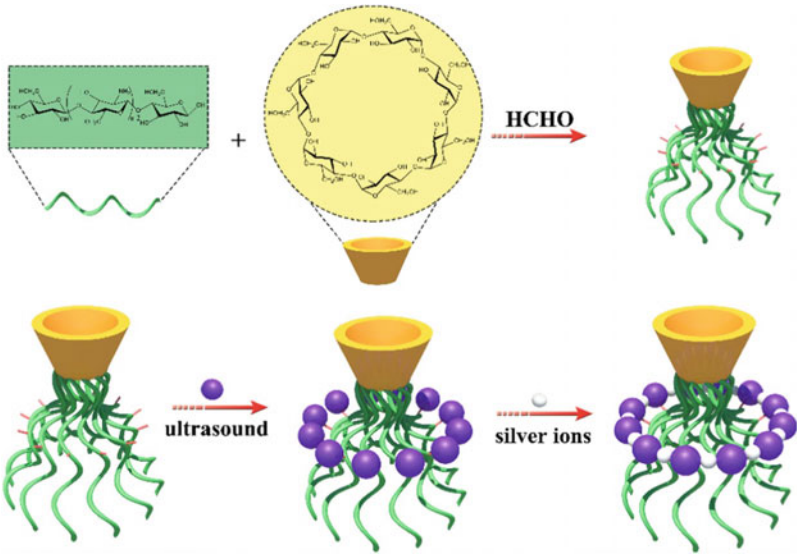


Fig. 2 Schematic diagram of the synthesis of SQD/CTSCD nanocomposite and the proposed mechanism for the detection of Ag^+ ions. Reproduced with permission from reference [44]

be broadly categorized into surface passivation, cation exchange, and aggregation-induced emission enhancement (AIEE).

2.2.1 Surface Passivation

QDs have surface defects such as interstitials, vacancies, and dangling bonds which act as trap sites for charge carriers, leading to the reduction of photoluminescence efficiency [45]. When the surface defects are passivated, the fluorescence becomes enhanced as the trap sites for the charge carriers are minimized on the surface of the QDs. Passivation in QDs has been realized when the QDs are used to detect biological molecules such as ascorbic acid [46]. Passivation of surface defects is also realized inorganically using ions such as defects by either Zn^{2+} or Cd^{2+} ions, which can also increase in the fluorescence lifetimes [47]. The size of the QDs determines the PL enhancement in that smaller QDs bear more surface defects than larger ones due to the large surface area-to-volume ratio. As such smaller QDs can accommodate larger concentrations of the metal ion to passivate the surface while larger ones may require lower concentration due to smaller surface area resulting in varied detection ranges [48]. Liu et al. [49] demonstrated that SnO_2 QDs exhibit an enhanced fluorescence intensity accompanied by high selectivity to Hg^{2+}. The fluorescent enhancement was observed because of the existence of dangling bonds due to the Sn vacancies on the QD surface which promote non-radiative recombination, resulting in low fluorescence efficiency that was passivated by Hg^{2+} which adsorbed onto the surface of SnO_2 QD to form extra bonds with the O atoms, and consequently eradicating the dangling bonds. Furthermore, the adsorption of Hg^{2+} on the SnO_2 QD surface created an electrostatic repulsion between the QDs and Hg^{2+}, which led to improved dispersion of SnO_2 QDs and weakened the self-quenching effect caused by aggregation and hence the enhanced fluorescence. In another study, Liu and colleagues [50] synthesized mercaptopropionic acid (MPA) capped Zn-doped $AgInS_2$ QDs composite that also displayed a linear increase in the fluorescent intensity with increased Cd^{2+} concentrations due to surface defect passivation (Fig. 3a), which was verified by the prolonged fluorescence lifetimes from 262.55 ns (in the absence of Cd^{2+}) to 292.33 ns (in the presence of Cd^{2+}) after the addition of 290 μM Cd^{2+}(Fig. 3b) [50].

2.2.2 Aggregation-Induced Emission Enhancement (AIEE)

The Aggregation-Induced Emission Enhancement (AIEE) mechanism was first reported by Tang's group in 2001 [51]. The group found that fluorophores in high concentrations exhibit strong fluorescence, mainly because in dilute solutions, the molecules rotate internally, while in aggregated form internal rotation is restricted due to space constraints thereby reducing the likelihood of non-radiative energy. Therefore, the excited QDs can only return to the ground state in the form of a radiative leap, resulting in enhanced fluorescence [51]. For example, Wang et al. [52]

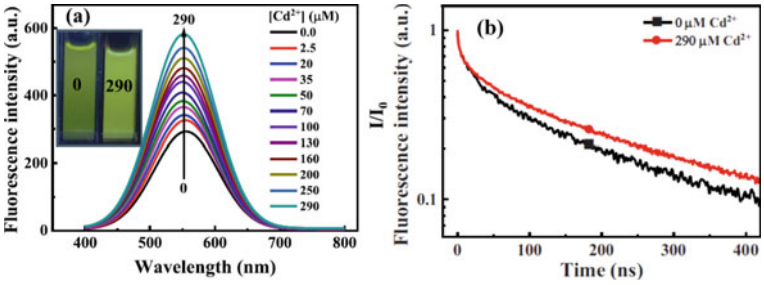


Fig. 3 PL **a** and time-resolved fluorescence spectra **b** of MPA-capped Zn-doped $AgInS_2$ QDs in the presence of Cd^{2+} ions at increased concentrations (0–290 μM). Reproduced with permission from reference [50]

synthesized glutathione (GSH) functionalized C-dots (g-CDs) to selectively detect Fe^{3+} in cells. The fluorescence intensity gradually increased with the increasing Fe^{3+} concentration. The absorbance peak at 433 nm showed an enhancement when Fe was 100 vol%, compared to the pure g-CDs in aqueous solution. The TEM micrographs of the g-CDs in the ethanol-water medium and in (Fe, 50 vol%) confirmed the well-dispersed QDs in the absence of Fe while an aggregation of the QDs was observed in the presence of 50% Fe, thus confirming the AIEE due to the agglomerated g-CDs.

This mechanism is also reported by Wei and colleagues [53] who displayed the detection of Cd^{2+} ions using L-cysteine-capped Zn-doped Ag-In-S (ZAIS) QDs composites. The L-cysteine ligands on the ZAIS composite surface coordinated with the Cd^{2+} via the thiol group which weakened the electrostatic repulsion between the composites encouraging aggregation. The reduction in the negativity charge of the ZAIS composite from −38.8 to −27.4 mV in the presence of Cd^{2+} confirmed the reduction of the repulsion efficiency in the system. In addition, the Cd-SR system passivated the surface defects of the ZAIS composite (ZAIS@Cd-R) thus decreasing the non-radiative recombination, which enhanced the PL intensity [53].

2.3 Cation-Exchange

Cation-exchange is the substitution of cations in an ionic crystal with guest cations while maintaining the original anionic structure and rarely changing the size [54]. Cation-exchange has been reported to result in either PL quenching or PL enhancement. The quenching or enhancement of the PL depends on the effect of the guest cation on the QD. A turn-on mechanism for the detection of Hg^{2+} was realized with Mn-doped ZnSe QDs composite. The Hg^{2+} ions replaced the Mn^{2+} in the Mn-doped ZnSe QDs composite, which eliminated the fluorescence quenching effect of Mn^{2+} (Fig. 4a, b) [55]. The ICP-AES revealed that before the addition of Hg^{2+}, the amount of Mn^{2+} in the doped QDs was 1.8% but was partially replaced by Hg^{2+} and its content decreased to 1.1%, while the content of Hg^{2+} increased from 0.0 to 1.4%. In

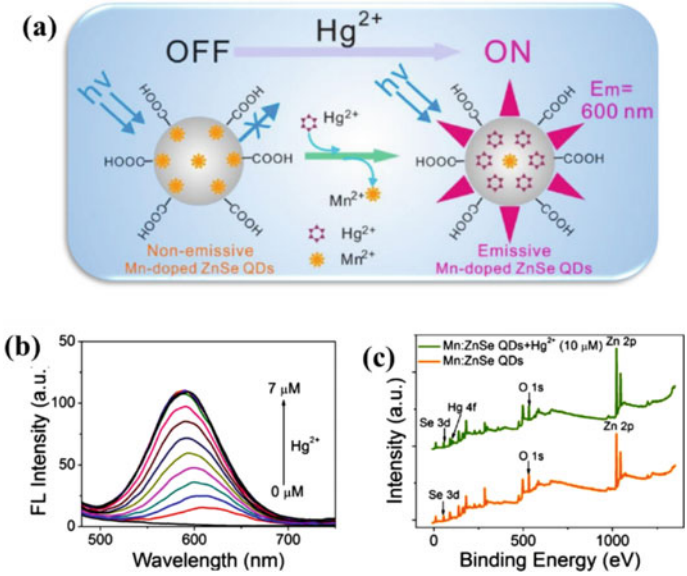


Fig. 4 **a** Schematic illustration of Mn-doped ZnSe sensor for Hg^{2+} detection. **b** Emission spectra of Mn-doped ZnSe QDs (40 μg/mL) upon the addition of different concentrations of Hg^{2+}, at an excitation wavelength of 400 nm. **c** XPS spectra of Mn-doped ZnSe QDs in the absence of Hg^{2+} and presence of 10 μM Hg^{2+}. Reproduced with permission from reference [55]

the absence of Hg^{2+}, the X-ray photoelectron spectroscopy (XPS) analysis showed the high-intensity characteristic peaks of Se, Zn, and O however, in the presence of Hg^{2+}, the intensity of the characteristic peak of Mn decreased (Fig. 4c), indicating the decrease of Mn^{2+} concentration on the surface of QDs, which is ascribed to the substitution of interfacial Mn^{2+} by Hg^{2+} [55].

In another development [56], a fluorescence quenching mechanism was attributed to the detection of Hg^{2+} ions using amino poly-(styrene-co-maleic anhydride) (NH_2-PSMA)-capped CdSe/ZnS core/shell QD nanocomposites due to the cation-exchange between Zn^{2+} on the ZnS shell and Hg^{2+} ions. This resulted in the formation of HgS particles on the CdSe/ZnS QD surface which destabilized the QD hence the quenching.

3 QD-Based Nanocomposites as Metal Sensors

QD-based nanocomposites are multi-functional composite materials that consist of QDs and one or more functional materials. The fabrication of QD-based nanocomposites is mainly aimed at enhancing the existing QD properties (i.e. optical, electrical, solubility, photostability), introducing other properties to the QDs (i.e. magnetic) and often achieved via core/shell structured and multi-material co-encapsulation

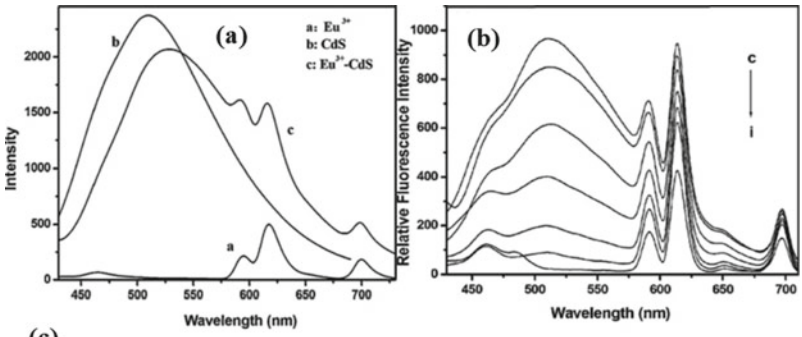
approaches. In addition, the incorporation of QDs into other functional materials creates opportunities to introduce new sites for anchoring guest molecules such as metal ions. This has resulted in numerous reports of the detection of heavy metal ions using QD-based nanocomposites. The following sections briefly discuss the use of various types of QD-based nanocomposites in heavy metal ion detection including metal-doped QD, QD-based polymeric, QD-based magnetic, QD-based MOFs, and QD-based bioconjugate nanocomposites.

3.1 Metal-Doped QDs Nanocomposites as Metal Sensors

QD doping is the intentional introduction of atoms to QDs to either improve or introduce additional properties to the existing QD material. Consecutively, QDs have been doped with metals to color-tune their emission spectra, improve fluorescence, electrical properties, and stability as well as introduce magnetic properties for enhanced efficiency in biomedical, environmental monitoring, and energy applications [57, 58]. In some cases, metal doping has been reported to occur unintentionally during shell growth on core QDs. Here, the metal dopant tends to replace some of the existing cations within the QD lattice (cation-exchange). For instance, many authors have reported the replacement of cations (i.e. Cu^+, Ag^+, In^{3+}) in ternary I–III–VI-type core QDs with Zn^{2+} ions during the passivation of the core QDs with ZnS shell, which resulted in an unexpected blue-shift in the fluorescence spectra [47, 59]. As a result, Guo et al. [53] presented a blue-shift emission inhibition approach during ZnS shell growth on $CuInS_2$ QDs by intentionally introducing Zn ions into the $CuInS_2$ core QDs during their synthesis (CuInSZn composite) to inhibit the cation-exchange process that occurs between Zn^{2+} and the cations (Cu^{2+}/In^{3+}) during ZnS shell formation [53].

Several authors have reported metal doping as an effective approach for improving the metal sensing ability of QD materials [31, 54, 55]. For example, Chen et al. [60] discovered a remarkable enhancement in the fluorescence peak of Eu^{3+} ions when glutathione (GSH)-capped Eu-doped CdS nanocomposites were produced, indicating a fluorescence energy transfer from the CdS QDs to the Eu^{3+} ions (Fig. 5a). The fluorescence peak of Eu^{3+} continued to enhance at increased concentrations of the Eu-doped CdS nanocomposites, promoting wider detection ranges and detection limits for Hg^{2+} via fluorescence quenching (Fig. 5b, c). The strong interaction between the amide (-NH_2) groups on the GSH-Eu-doped CdS nanocomposite and the Hg^{2+} could be responsible for the electron transfer from the QD nanocomposite to Hg^{2+} which resulted in the quenching. Furthermore, the fluorescence lifetime of the nanocomposite gradually decreased at increased Hg^{2+} ions from 512.19 to 488.54 and 437.56 ns (500 nmol/L and 1200 nmol/L) respectively, which confirmed the enhanced electron transfer process at increased Hg^{2+} concentrations [60].

GSH-capped Zn-doped $CuInS_2$/ZnS (CuInZnS/ZnS) core/shell nanocomposites displayed fluorescence quenching due to mild cation-exchange during the detection of Cu^{2+} ions in aqueous solutions [61]. The cation-exchange was attributed to the



(c)

Concentration of Eu^{3+}-doped CdS with Cd^{2+} as a standard (mol L^{-1})	Linear range (nmol L^{-1})
0.1×10^{-4}	3–250
0.5×10^{-4}	5–650
2.0×10^{-4}	10–1500
3.5×10^{-4}	90–2000
5.0×10^{-4}	500–4500

Fig. 5 PL spectra of Eu^{3+}, CdS, Eu-doped CdS (**a**), and Eu-doped CdS with different concentrations of Hg^{2+} (c–i is 0, 60, 300, 500, 700, 900, and 1500 nmol/L, respectively) (**b**). Produced linear detection range for Hg^{2+} at increased concentrations of the Eu-doped CdS QD nanocomposite (**c**). Reproduced with permission from reference [60]

replacement of Zn^{2+} with Cu^{2+} ions which was also reflected in the red-shifted fluorescence and absorbance spectra probably due to some size changes in the wider band gap of ZnS after the cation-exchange process. The cation-exchange was confirmed by the decline in the In and Zn concentrations of the CuInZnS/ZnS nanocomposite from 55.0% to 54.3% and 37.55 to 33.7% at increased Cu^{2+} concentrations, respectively. The time-resolved PL spectra revealed a decrease in the fluorescence lifetimes of the nanocomposite in the presence of Cu^{2+} ions from 413 to 379 ns and 289 ns at 10 μM and 100 μM, respectively suggesting a gradual change in the electron–hole recombination process during emission at increased Cu^{2+} concentrations. The CuInZnS/ZnS nanocomposite produced a linear range and DL of 0.020–20 μM and 6.7 nM for the detection of Cu^{2+} ions. Good performance was achieved towards real water samples compared to the accuracy of the ICP-atomic emission spectroscopy (AES) method [61].

3.2 QD-Based Polymeric Nanocomposites as Metal Sensors

The QDs-based polymeric nanocomposites are fabricated by combining a QD/nanofiller with a polymer matrix [62]. The incorporation of QDs in a polymer is attempted in various ways, such as (i) ligand-exchange between polymer and QDs;

(ii) grafting polymer to QDs; (ii) grafting polymer from QDs; (iv) capping polymer onto QDs; and (iv) growing QDs within the polymeric template. The polymer encapsulation of QDs not only offers protection to the QD and sometimes enhances the optical properties and photo stability of the QD but also provides functional groups that target molecules such as heavy metal ions for their detection [63, 64].

For example, Zhou et al. [56] reported aminolyzed poly-(styrene-co-maleic anhydride) (NH_2-PSMA)-capped CdSe/ZnS QD nanocomposites for the detection of Hg^{2+} and Cu^{2+} ions via fluorescence quenching mechanism [56]. The fluorescence quenching of the NH_2-PSMA-capped CdSe/ZnS QD upon the addition of Hg^{2+} was attributed to the formation of HgS particles on the CdSe/ZnS QD surface due to the cation-exchange between Zn^{2+} and Hg^{2+} ions. The formation of the HgS particles generated surface defects which destabilized the QD hence the quenching. The formation of HgS particles was evidenced by the disappearance of the NH_2-PSMA-capped CdSe/ZnS QD absorbance peak, the red-shifted fluorescence peak, and the color change from colorless to yellow in the presence of Hg^{2+} ions. On the contrary, the fluorescence quenching of the NH_2-PSMA-capped CdSe/ZnS QD upon the addition of Cu^{2+} was attributed to the electron transfer from the NH_2-PSMA-capped QD to Cu^{2+} via the co-ordination of Cu^{2+} with the amide, hydroxyl, and carbonyl groups on the PSMA, which formed non-radiative surface channels. No changes were observed in the absorbance, fluorescence spectra, and solution color of the NH_2-PSMA-capped CdSe/ZnS QD in the presence of Cu^{2+}, indicating that no new molecules were formed. Both Hg^{2+} and Cu^{2+} ions could be detected in co-existence in the presence of a masking agent. Linear detection ranges of 0.02–0.7 μM (LOD = 6.94 nM) and 0.1–1.4 μM (LOD = 20.58 nM) were achieved for Cu^{2+} and Hg^{2+} ions, respectively [56].

In another development, He et al. [65] reported the simultaneous detection of Ag^+, Hg^{2+}, Cu^{2+}, and Fe^{3+} ions using a QD-polymeric nanocomposite based on the encapsulation of two hydrophobic green and red fluorescing QDs (Zn–CdSe/ZnS QDs_{523} and QDs_{600}) with a blue fluorescent amphiphilic block co-polymer, based on phenolphthalin grafted polyarylene ether nitrile (P-PEN). The detection of all the metal ions occurred via the fluorescence quenching of the P-PEN@Zn–CdSe/ZnS QDs_{523} and QDs_{600} nanocomposites. The fluorescence quenching that occurred in the detection of Ag^+ and Hg^{2+} was attributed to the generation of surface defects due to the cation-exchange reaction between Zn^{2+} on the ZnS shell and Ag^+/Hg^{2+} ions. The fluorescence quenching effect of Cu^{2+} was related to the combination of surface defect formation due to the cation-exchange between Zn^{2+} and Cu^{2+} as well as the coordination of Cu^{2+} with carbonyl groups on the P-PEN polymer. Similarly, to Cu^{2+}, Fe^{3+} ions coordinated with P-PEN polymer on the QD surface via carbonyl groups, and no evidence of cation-exchange between Zn^{2+} and Fe^{3+} was seen due to the lower Kpa of FeS (17.2) compared to that of ZnS (23.8) as observed in the detection of Hg^{2+}, Ag^+, and Cu^{2+} which exhibited Kpa's of 51.8, 49.2 and 35.2, respectively. The selective detection of metal ions was based on selective fluorescence quenching of the different-sized QDs within the nanocomposite, such that: Ag^+ and Hg^{2+} ions mainly quenched the green and red fluorescent QDs whereas high concentrations of Cu^{2+} and Fe^{3+} ions quenched the green, red fluorescent QDs and the blue fluorescent band of

the P-PEN polymer. The selectivity of Ag^+ and Hg^{2+} ions could be further observed in that the red fluorescent QD was quenched at a higher degree by the Ag^+ than the green fluorescent QDs while Hg^{2+} quenched the red and green QDs at a similar degree. In the case of Cu^{2+}, a higher quenching degree was evident in red fluorescent QD compared to blue and green. Additionally, Fe^{3+} ions revealed similar quenching degrees for all three fluorescent peaks, however, a higher quenching degree on the blue fluorescent band was observed compared to other metal ions which were related to the detection mechanism based on the coordination with the P-PEN via carbonyl groups (Fig. 6). Linear detection ranges of 0.1–1, 1–5 and 0.05–3, 3–5 μM were achieved for Ag^+ and Hg^{2+} for the green and red quenched fluorescence while 1–10, 10–50, and 0.5–10, 10–100 μM was achieved for Cu^{2+} and Fe^{3+} for the quenched fluorescence of all three emission peaks (Fig. 7). The P-PEN@Zn–CdSe/ZnS QDs_{523} and QDs_{600} nanocomposites showed potential in the simultaneous detection of Ag^+, Hg^{2+}, Cu^{2+}, and Fe^{3+} in environmental wastewater samples via spiked recoveries. Naked–eye detection of Ag^+, Hg^{2+}, Cu^{2+}, and Fe^{3+} using test strips was also demonstrated [65].

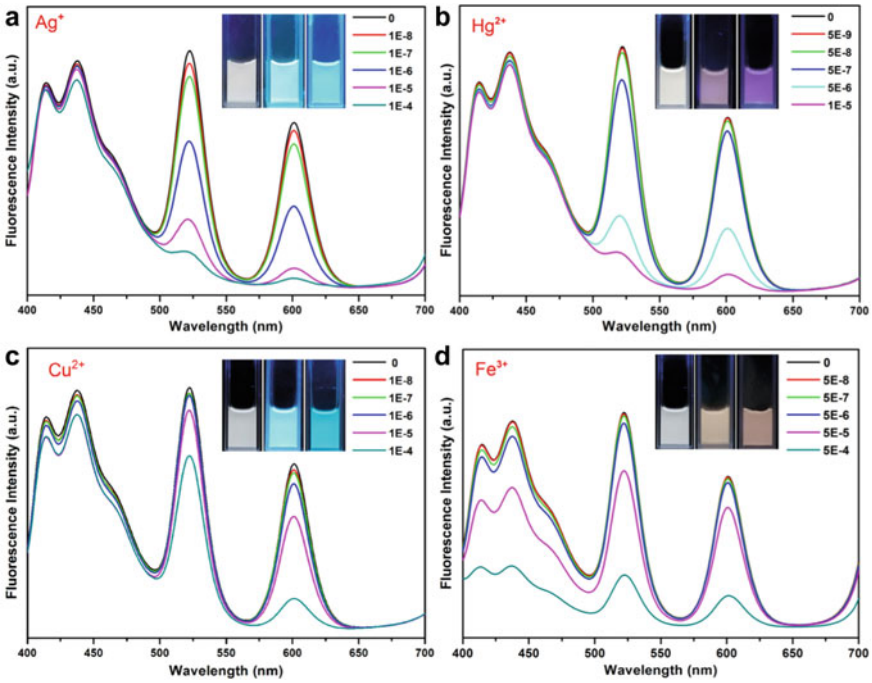


Fig. 6 PL spectra of P-PEN@Zn–CdSe/ZnS QDs_{523} and QDs_{600} NC in the presence of Ag^+ (**a**), Hg^{2+} (**b**), Cu^{2+} (**c**), and Fe^{3+} (**d**) (mol/L). Reproduced with permission from reference [65]

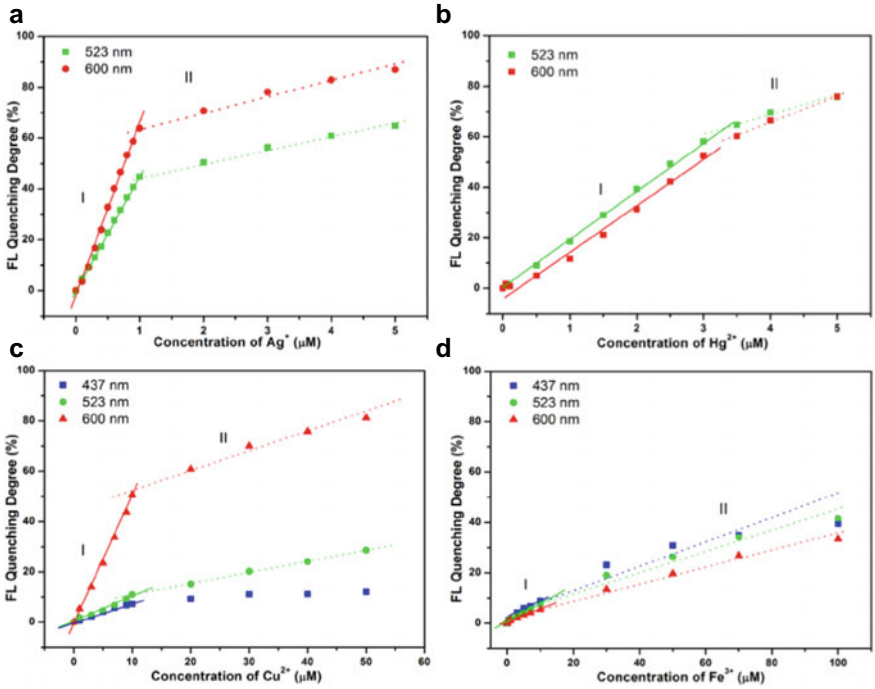


Fig. 7 Linear plot of fluorescence quenching degree of P-PEN@Zn–CdSe/ZnS QDs_{523} and QDs_{600} NC in the presence of Ag^+ (**a**), Hg^{2+} (**b**), Cu^{2+} (**c**), and Fe^{3+} (**d**) (mol/L). Reproduced with permission from reference [65]

3.3 QD-Based Magnetic Nanocomposites

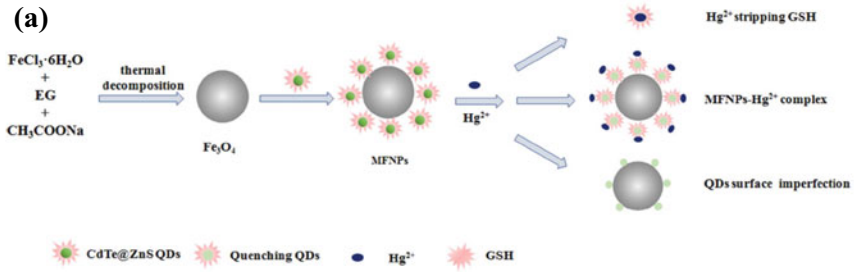
Magnetic QD nanocomposites are bi-functional (magneto-fluorescent) materials that are a result of combining magnetic nanoparticles (MNPs) such as iron oxide (Fe_3O_4) NMs and QDs. Magnetic QD nanocomposite has been mainly achieved by (i) co-encapsulation of individually synthesized MNPs and QDs in amphiphilic polymers or silica shell and (ii) core/shell formation where the QDs are deposited around the MNP to form a shell or vice versa [66].

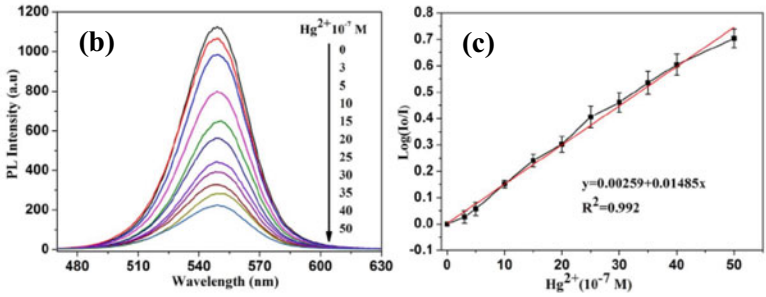
Magnetic QD nanocomposites have mainly benefited biological applications due to their excellent performance in biological imaging, drug delivery, and medical diagnosis [67, 68]. Until recently, researchers explored the bi-functional ability of magneto-fluorescent Fe_3O_4 NM-QDs nanocomposites (NC) in the fluorescence detection and removal of heavy metals in water [69–72]. The use of magnetic Fe_3O_4 NM as a magnetic component in the nanocomposites was motivated by their popularity in the removal of heavy metals in water due to their ease of separation and high adsorption capabilities [73–75], especially those with layered adsorptive structures such as functionalized meso-porous materials (e.g. SiO_2 and $CaCO_3$) [76–78]. Indeed, magneto-fluorescent Fe_3O_4 NM-QDs NCs based on carbon NM remain the

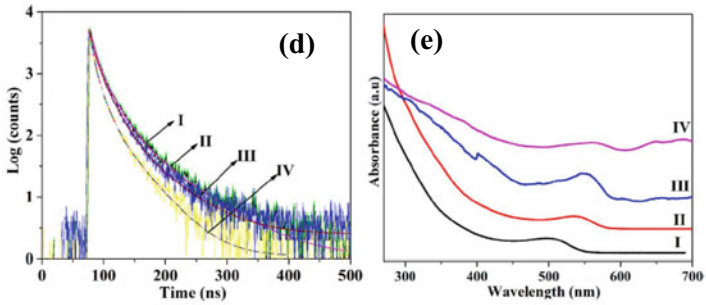
most popular in fluorescence detection and removal of heavy metals in water [79, 80], compared to those based on II–VI and I–III–VI QDs [71, 72, 81, 82].

For example, Alvand et al. [69] reported the synthesis of a multifunctional nanocomposite by the coating of the Graphene QDs (GQDs) onto the surface of amine (NH_2)-functionalized Fe_3O_4@SiO_2 nanospheres through acylamide binding in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) as the activator, which exhibited fluorescence detection and removal functions towards Hg^{2+} ions in aqueous solutions. The study reported a linear range of 0.1–70 μM and a LOD of 30 nM for the fluorescence detection of Hg^{2+} ions. Selective fluorescence quenching was achieved towards Hg^{2+} ions in aqueous solutions over other metal ions. However, the fluorescence was slightly quenched by Fe^{2+} and Fe^{3+} ions, thus the material can be used in the detection of Hg^{2+} ions after removal of these ions. The selectivity was based on the stronger interaction between carboxylic and hydroxyl functionality on the G QD surface and the Hg^{2+} ions over metal ions. Thus, the fluorescence quenching was attributed to the electron transfer from the G QDs to the Hg^{2+} ions, which facilitated non-radiative electron/hole recombination, suggesting dynamic quenching. The authors also suggested a probable combination of static and dynamic quenching at higher Hg^{2+} ions concentrations (>70 μM) due to deviation in the linearity of the Stern-Volmer plot. The detection method was successfully applied for the detection of Hg^{2+} in real water samples. The nanocomposite demonstrated a rapid intake of Hg^{2+} ions within 1.5 min and a maximum adsorption capacity of 68.03 mg/g. The reusability studies showed that the nanocomposite could be used for four cycles with negligible loss of detection [69].

A novel magnetic-fluorescent nanocomposite based on carboxymethyl chitosan (CMCN)-functionalized Fe_3O_4 NPs conjugated to GSH-capped CdTe/ZnS core/shell QDs (CMCN-Fe_3O_4@CdTe/ZnS) was created for the simultaneous determination and removal of Hg^{2+} in water (Fig. 8a) [83]. Under ideal circumstances, the fluorescence of CMCN-Fe_3O_4@CdTe/ZnS nanocomposite was quenched at increased concentrations of Hg^{2+} ions. Thus, a linear range and detection limit of 0.3–5 μmol/L and 9.1 nmol/L was achieved for the detection of Hg^{2+} (Fig. 8b, c). The fluorescence quenching was attributed to the formation of a non-fluorescent ground-state CMCN-Fe_3O_4@CdTe/ZnS-Hg complex, which occurred via coordination of Hg^{2+} with COO−, $-NH_2$, and OH^- groups on the CMCN nanocomposite surface, suggesting static quenching. Additionally, the Hg^{2+} ions compete with the GSH-capped CdTe/ZnS QD for GSH functionalization and win (Hg^{2+} striping), leaving an imperfect QD surface hence the fluorescence quenching (Fig. 8a). Static quenching was confirmed by the unchanged fluorescence lifetime in the presence and absence of Hg^{2+} ions (33 ns) (Fig. 8d, f). Furthermore, the red-shifted and enhanced absorbance band (Fig. 8e) observed after the addition of Hg^{2+} to CMCN-Fe_3O_4@CdTe/ZnS could be due to the formation of a non-fluorescent ground-state CMCN-Fe_3O_4@CdTe/ZnS–Hg complex. Co-existing ions (Na^+, K^+, Ca^{2+}, Mg^{2+}, Al^{3+}, Ba^{2+}, Zn^{2+}, Fe^{2+}, and Fe^{3+}) showed low interference effect in detecting Hg^{2+} ions however, Ag^+, Pb^{2+}, and Cu^{2+} ions showed a higher quenching effect, which was attributed to their affinity for COO^-, $-NH_2$ and OH^- groups on exposed nanocomposite surface [83].







(f)

Sample	B_1(%)	τ_1(ns)	B_2(%)	τ_2(ns)	B_3(%)	τ_3(ns)	τ(ns)	F_0/F	τ_0/τ
I	26.17	3.99	56.97	13.22	16.86	53.84	33.56	1	1
II	23.74	6.62	62.33	18.86	13.93	58.95	33.25	1.36	1.01
III	23.35	6.14	61.65	18.09	16.50	55.49	33.05	2.0	1.01
IV	26.77	6.81	54.12	20.73	16.11	53.36	32.52	2.88	1.03

The concentration of Hg^{2+} (10^{-7} mol/L)(I) 0, (II) 10, (III) 20 and (IV) 30.

Fig. 8 Schematic diagram of the synthesis CMCN-Fe_3O_4@CdTe/ZnS nanocomposite for the detection of Hg^{2+} (**a**). PL spectra (**b**), linear fluorescence intensity plot (**c**), time-resolved fluorescence spectra (**d**), absorbance spectra (**e**), and fluorescence lifetimes (**f**) of CMCN-Fe_3O_4@CdTe/ZnS nanocomposites in the presence of Hg^{2+} (1×10^{-7} M–50×10^{-7} M). Reproduced with permission from reference [83]

3.4 QD-Based MOFs Nanocomposites as Metal Sensors

MOFs exhibit excellent properties such as ring structures, the cores of which are capable of accommodating smaller molecules, atoms, or ions of smaller dimensions, thus serving as hosts to guest materials [84, 85]. The functional groups on the surface or within the MOFs can serve as the sites for anchoring the guest molecules/materials such that the synergetic effect of both materials can be exploited. The ability of the individual MOF's materials to interact with one another via covalent and non-covalent means such as hydrogen bonding and π–π assembling makes MOFs unique materials to be reckoned in the first place. As a result, many MOFs have been discovered and exploited for various applications, including sensing, hydrogen evolution reactions, ammonia production, and carbon dioxide reduction.

It is obvious that by combining the catalytic, optical, and optoelectronic properties of the QDs with the larger size dimension, and functionality such as catalytic, optical, photoluminescence, phosphorescence, light harvesting, and utility, the high reaction surface area, ring morphology, and pore volume sizes of the MOFs, new hybrid materials with better performance for sensing, imaging and energy utility can be achieved. However, to achieve this interesting objective, a look into the synthesis procedures for both QDs and MOFs is essential.

Typically, QDs are nanomaterials whose size dimension falls between 1 and 10 nm. Thus, all techniques capable of achieving this size dimension are taken into consideration, viz: hydrothermal, precipitation, carbonization, and a combination of a number of these approaches [86, 87]. On the other hand, MOFs are materials whose size can be reduced from macro-to-micro and micro-to-nanoscale. Thus, forming QDs-MOFs systems may involve the following strategies: (i) synthesizing separately the QDs and MOFs and afterward combining them under suitable conditions that will not tamper with their structures and integrity or (ii) synthesizing through one pot, in which all precursors are placed in the reaction vessel at the same time and cause to undergo the same transformation conditions, e.g., heating, refluxing, mixing, etc. Both strategies have been successfully utilized for making excellent QDs-MOFs with profound performance [6].

Due to the synergetic effects of their individual material properties, the effectiveness of using QDs-MOFs as efficient sensing agents for heavy metal ions has been explored. For example, Wang et al. [88] produced QD@MOF based on zeolitic imidazolate framework (ZIF) and CdTe QDs. The CdTe@ZIF-365 was utilized as a fluorescent ratiometric sensor for Cu^{2+} with high sensitivity and selectivity in the mixed cation ions' solution (Fig. 9a, b). Here, the fluorescence detection of the Cu^{2+} was mainly based on the quenching of the CdTe QDs fluorescence peak since the ZIF-365 fluorescence was only slightly quenched. A good linear relationship was established between the fluorescence intensity of the CdTe@ZIF-365 nanocomposite and Cu^{2+} ion concentration (0 0.0075 μmol/L) with a detection limit of 0.25 nM (Fig. 9c, d) [88].

Furthermore, Guo et al. [89] reported the use of QD@MOF based on the co-encapsulation of carbon (C) QDs and Au nanoclusters (NCs) with ZIF-8 (CQD/

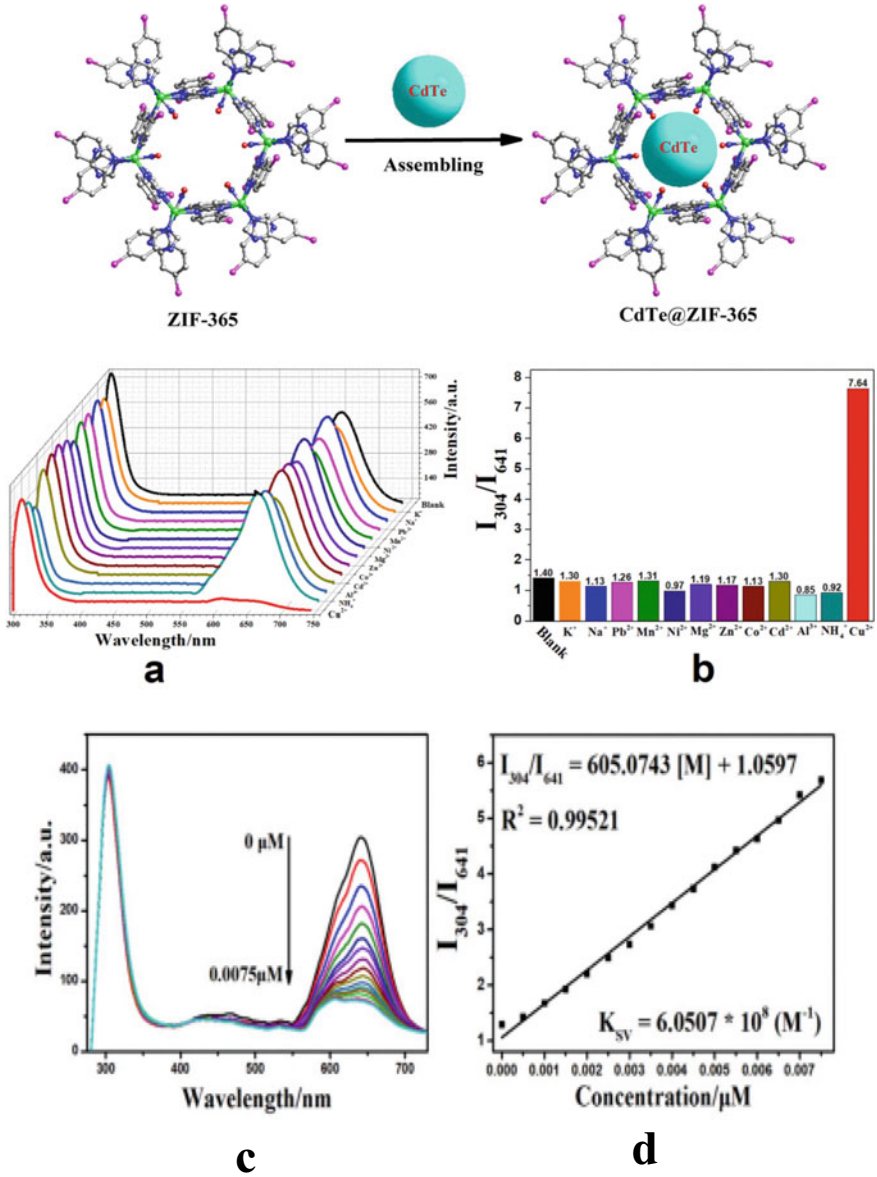


Fig. 9 Schematic diagram of the fabrication of CdTe@ZIF-365 NC (top). PL spectra and fluorescence intensity ratio (304 nm/641 nm) of CdTe@ZIF-365 NC in the presence of other metals (**a**, **b**). PL spectra and ratiometric plots (**c**, **d**) of CdTe@ZIF-365 NC in the presence of Cu^{2+} ions. Reproduced with permission from reference [88]

Au-NC/ZIF-8) for the ratiometric fluorescence detection of Hg^{2+} ion in an aqueous medium. The dual-emission originated from the fluorescence of the C QDs (440 nm) and the Au NCs (640 nm). The fluorescence peak at 640 nm was gradually quenched at increased Hg^{2+} concentrations while that at 440 nm showed no obvious changes in fluorescence intensity. Thus, a linear range and detection limit of 3–30 nM and 1 nM was established based on the quenched fluorescence peak of Au NCs. The continuous quenching at 640 nm and unchanged fluorescence at 440 nm at increased Hg^{2+} concentrations was also reflected in the fading red emission and final blue emission. There was a neglectable change in the fluorescence lifetime after adding Hg^{2+} to the CQD/Au-NC/ZIF-8, which confirmed the quenching was due to a static process. The selectivity studies showed a neglectable change in fluorescence intensity and emission color when CQD/Au-NC/ZIF-8 was exposed to other metal ions, indicating that the nanosensors were selective to Hg^{2+} ions [89].

3.5 QD-Based Bioconjugates Nanocomposites as Metal Sensors

The development of biocompatible NPs for bioimaging and biosensing is an area of considerable current interest in research [90]. In broad biological research, where conventional fluorescent labels based on organic molecules fall short of providing long-term stability and simultaneous detection of numerous signals, the distinctive optical features of QDs make them appealing as *in-vivo* and in-vitro fluorophores. QDs can be conjugated with biomolecules such as peptides, deoxyribonucleic acid (DNA), nucleic acids, and proteins to produce bioconjugated QD nanocomposites [91, 92]. Thus, the QD acts as a nano-scaffold for attachment to biomolecules, creating a multifunctional nanoparticle–biological hybrid. Some of the conjugation techniques utilized to attach biomolecules to the surface of QDs are direct adsorption onto the quantum dot surface and the application of inert polymer coatings [93, 94]. Any modification to a QD's outer surface must meet the following criteria: (1) make the QDs water-soluble; (2) make them biocompatible; (3) add surface reactive groups for conjugation; (4) keep all QDs' advantages in terms of photochemical long-term stability (spectral range, brightness, fluorescence, narrow emission); (5) produce monodispersed particles or particles with a narrow size distribution; and (6) add a thin shell and capping layer to prevent the engineered particle from getting bigger [95, 96].

QD-bioconjugate nanocomposites have been used to label, image, and detect a variety of materials, from large proteins and nucleic acids to metal ions and tiny molecules [5, 97, 98]. Both fluorescent turn-on and turn-off sensing techniques have been used for both biomolecule analysis and extracellular/ intracellular metal ion detection [5]. The sensitive detection of intracellular metal ions is important to understand biological functions and for various medical diagnoses. For instance, Zn is the second most abundant metal in the human body, essential for several biological

processes but can be toxic when present excessively. Unusual Zn^{2+} patterns have been associated with Prostate cancer. Alzheimer's disease, diabetes, and epilepsy. Several QD-based nanocomposites have been explored for intracellular metal ion sensors [5, 99], but only a few reports are based on QD-bioconjugates. For example, QD-based Bi-FRET biopolymeric nanocomposite containing two colored QDs (517 nm and 560 nm), were fabricated for ratiometric sensing of Zn^{2+} in different media [4]. The QD scaffold was coated with poly(dA) homopolymer/double-stranded DNA, followed by the loading of meso-tetra(4-sulfonatophenyl)porphine dihydrochloride (TSPP). The BiFRET process is based on FRET-1, where an efficient overlap between QD 517 nm emission and TSSP absorption band at 515 nm occurs and in FRET-2 the overlap is inefficient due to the weak absorption peak at 555 nm. During Zn^{2+} sensing, Zn^{2+} chelates with TSPP, resulting in the reduction of the 515 nm absorption peak and enhancement of the 555 nm absorption peak. This causes a reduction in FRET-1 and an enhancement in FRET-2 (Fig. 10a, b). Consecutively, the QD-based Bi-FRET biopolymeric nanocomposite produced a linear detection range and detection limit of 0.05–4 μM and 1 nM (Fig. 10c). The nanocomposite was applied for sensing Zn^{2+} ions in solution or paper substrate and to visualize Zn^{2+} in living cells [4].

In another development, Wu et al. [99] demonstrated the use of DNAzymes coupled SiO_2@CdSe/ZnS QDs as selective sensors for Pb^{2+} and Cu^{2+} ions in aqueous solutions. The DNAzymes were made from two quenchers and one of the quenchers was designed with binding pockets that are selective to Zn^{2+} and Cu^{2+} ions. The sensing mechanism was based on the fluorescence turn-on of the QD upon the binding of metal ions to the DNAzymes due to the cleavage of DNAzymes, which separates from QD (Fig. 10d). Multiplexed detection of Cu^{2+} and Zn^{2+} was demonstrated by using two QDs of different emission colors within a range of 0.2–4 μM with detection limits of 0.2 nM and 0.5 nM, respectively [99].

4 Challenges, Conclusion, and Future Perspectives

For years, new functional materials based on QDs have been created and improved for analytical and bioanalytical applications. One of the realized shortcomings associated with QD-based nanocomposites is their fabrication. For instance, QDs-polymer composites suffer from problems associated with the compatibility of the polymer and the QD as well as obtaining proper homogeneity of QDs in the polymer host. In some cases, the QD nanocomposite loses its optical properties during the incorporation of QDs with magnetic materials and paramagnetic ions. Even though a lot of research on synthesis methods has been published in the previous decade, it is still challenging to discover a scalable, effective, but straightforward manufacturing pathway to produce QD nanocomposites. Another shortcoming is achieving selective detection of metals in the midst of other molecules and multi-analyte detection. Although many attempts have been successful with varied functionality, vast surface area, up-conversion photoluminescence, and the electron reservoir features of QDs,

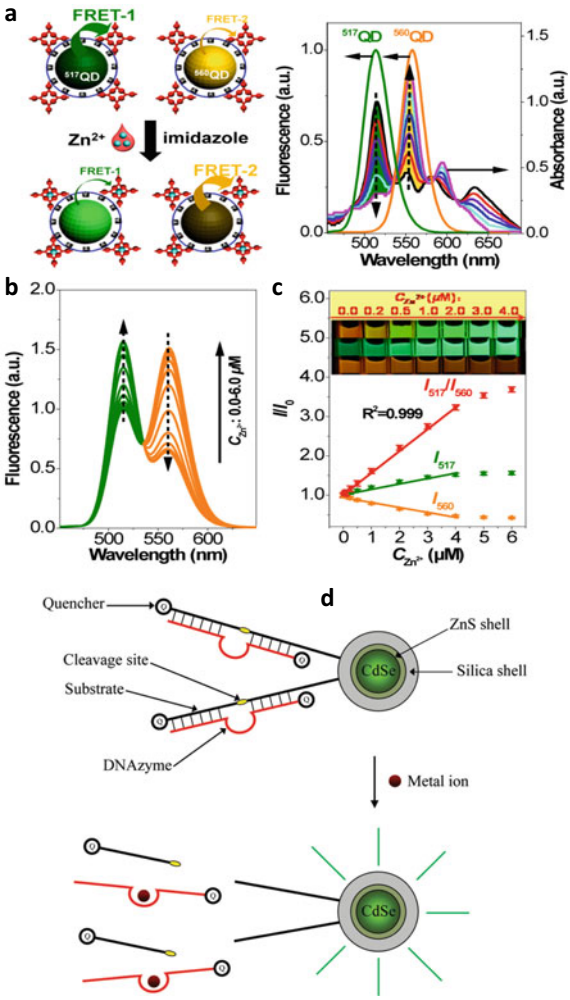


Fig. 10 Schematic diagram demonstrating the detection of Zn^{2+} ions using the QD-based Bi-FRET biopolymeric nanocomposite (left) and the corresponding spectral modeled response (right) (**a**). PL spectra **b** and linear plot of fluorescence intensity ratios (QD_{517}/QD_{560}) **c** of QD-based Bi-FRET biopolymeric in the presence of Zn^{2+} ions (0.2–4 μM). [99] Reproduced with permission from reference [4]. **d** The schematic diagram for the sensing mechanism of Pb^{2+} and Cu^{2+} ions using DNAzymes@SiO_2@CdSe/ZnS QDs. Reproduced with permission from reference

this field of research could benefit from tapping into molecular imprinting strategies and the incorporation of more than one QD type to target different analytes at once.

Metal detection is mostly accomplished by the interaction of QDs, however, the introduction of other functional materials to the QDs has demonstrated great potential in pollutant removal especially those based on carbon and magnetic nanomaterials. Due to their unmatched flexibility and integration for multi-analyte detection, these quantum dots nanocomposites have created a wide range of intriguing possibilities in environmental and biological sensing applications.

With the use of targeted ligands such as nucleic acids, peptides, antibodies, and small molecules, QD synthesis, and functionalization continues to improve. Studies on QD-based intracellular sensors are advancing their potential use due to their great

biocompatibility and performance in super-targeted administration. However, QD technology still has considerable difficulties achieving the intended broad bioanalytical goals. The difficulties include some restrictions, such as the size compatibility, which restricts their tissue penetration; low stability (low quantum efficiency and high decay rates); as well as the toxicity consequences. We think that a major factor in their extensive use is the development of more efficient, reliable, and repeatable conjugation techniques. Looking ahead to QD technology, there is tremendous bioanalytical potential, and as soon as these limiting issues have been addressed, the field will make a huge advancement.

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Quantum Dots Composites in Catalysis Applications

Krishna Hari Sharma, Nallin Sharma, and Chandan Srivastava

Abstract The quantum dot (QD) has displayed numerous outstanding roles in scientific advancements recently. Another aspect of QD development is their role in photocatalytic activity. The optical tunnability of QD-based composites has immensely attracted the scientific community to explore their capabilities. The various studied compositions are in accordance with carbon nanomaterials, transition metal oxide nanomaterials, metal chalcogenides nanomaterials, perovskites nanomaterials. These three are broader categories covered in the chapter with an interest towards various photocatalytic approaches.

Keywords Quantum dots · Composites · Catalysis · Charge-transfer

List of Abbreviations

CQDs	Carbon QDs
MX	Chalcogenides
ChQDs	Chalcogenides QDs
CB	conduction band and valence band (VB)
COFs	covalent organic frameworks
CTF-1	covalent triazine-based frameworks
EHP	electron-hole pair
g-C3N4	graphitic carbon nitride
MPA	mercaptopropionic acid
MOFs	metal-organic frameworks

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N-CQDs	nitrogen-doped CQDs
PQDs	Perovskite QDs
QD	Quantum Dots
rGO	reduced graphene oxide
SQDs	Semiconductor QDs
HNTs	silica/halloysite nanotubes
TC-HCl	tetracycline chloride
TGA	thioglycolic acid

1 Introduction

The Industrial Revolution expedited the growth of economic development which hauled the prosperity of humankind. However, the backbone of this opulence is fossil fuels for energy production. It is predicted that the energy consumption globally will reach around 25–27 TW, by 2050 [1–3]. Moreover, such heavy reliance on fossil fuels leads to severe problems such as the exhaustion of greenhouse gases (causing air pollution), water pollution (freshwater streams, seawater), and soil pollution (because of landfills). To foresee a better future for the next generation, a better alternative is required for the production of clean energy, and to revive the natural resources. To deal with this, the scientific community seeks immense hope in the catalysis process. The catalysis process facilitates the production of clean fuel (such as H_2), wastewater treatment (using photo-and electro-catalysis), and VOC abatement [4–7]. The applications of QD in photocatalytic reaction producing Hydrogen and oxygen are of keen interest to the scientific community. Hydrogen evolution reaction (HER) and Oxygen evolution reaction (OER) are being studied with prime emphasis in several atmospheric conditions. An efficient catalyst can be engineered by fabricating a hybrid composite containing semiconductors, metal nanoparticles, quantum dots etc. [8]. Among all the materials, quantum dots (QDs) are the focus of the interest in this chapter which play pivotal roles in designing efficient composites for catalytic applications. Being quasi-zero-dimensional materials, QDs possess the large surface area which makes them capable of rapid electron transfer, tunable chemical activity, renewability, nontoxicity, large two-photon absorption cross-sections, up-conversion ability, and excellent selectivity [9, 10]. Therefore, the performance of QDs-based composites can be modulated by modifying their surface and bulk features, and energy gap enlargement [11]. These properties make QDs an excellent candidate for catalytic applications. This chapter features the role of carbon QDs-, semiconductor QDs-, and perovskite QDs-based composites for their applications in catalytic based energy conversion processes.

2 Carbon QDs (CQDs)-Based Composites

Carbon-based materials, as of its abundance in nature, have always attracted researchers and scientists for their applications in catalysis. In addition, these materials are bio-compatible, less toxic, photostable, and have the ability of chemiluminescence, multiphoton excitation, and up-conversion photoluminescence [12–14]. Moreover, carbon QDs (CQDs), being a member of carbon family, inherits all the capabilities. Besides, CQDs also possess larger surface area, high conductivity, and supernal charge-transfer capabilities. The electronic properties of the CQDs can further be modified using the doped heteroatom (B, N, P, S, etc.), this strategy enhances the catalytic properties via intramolecular charge transfer [15, 16]. Simultaneously, the intermolecular charge transfer can also boost by the CQDs-based composites. Based on the synthesis precursors, different functional groups ($-NH_2$, $-OH$, $-COOH$, etc.) present on the surface of CQDs could provide favorable sites for highly active catalysis processes [17].

There are several approaches which should be in considerations while fabricating an efficient CQDs-based composite. Here, some of them will be discussed. To begin with, the use of CQDs as an active center to promote defect sites for catalytic reactions-CQDs/graphene composite was fabricated to achieve the overall water splitting [18]. In this work, the combination of CQDs and graphene facilitated the catalytic reaction in synergy-CQDs offer abundant active edges and defect sites for electrocatalytic reactions. Whereas, the graphene served as a conductive substrate which prevent the aggregation of CQDs, and ease the charge transfer process (Fig. 1).

Using another approach, the composites of CQDs and metal compounds can be used as an efficient catalyst. Generally, the abundance presence of functional groups on the surface of CQDs encourages the coordination between CQDs and metal ions with empty d-orbitals. This leads to the formation of stable CQDs-metal compounds composites [19]. Moreover, the employed CQDs boast to form ultrafine crystals with stable nanostructures [20]. In another report, it was reported that the nitrogen-doped CQDs (N-CQDs) helps to improve the surface area of MnO_2 nanoflowers

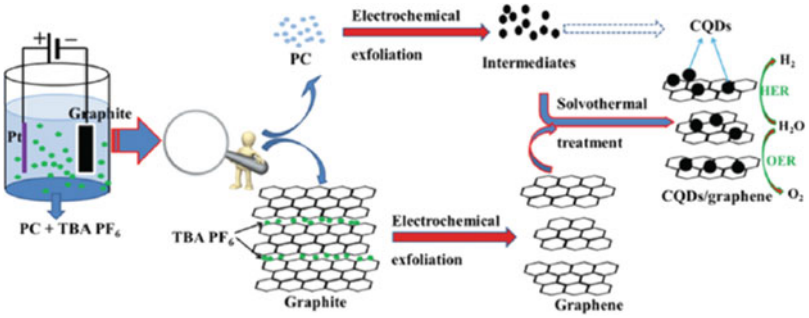

Fig. 1 Probable formation route of the CQDs/graphene heterostructure [18]. Copyright 2019, Royal Society of Chemistry

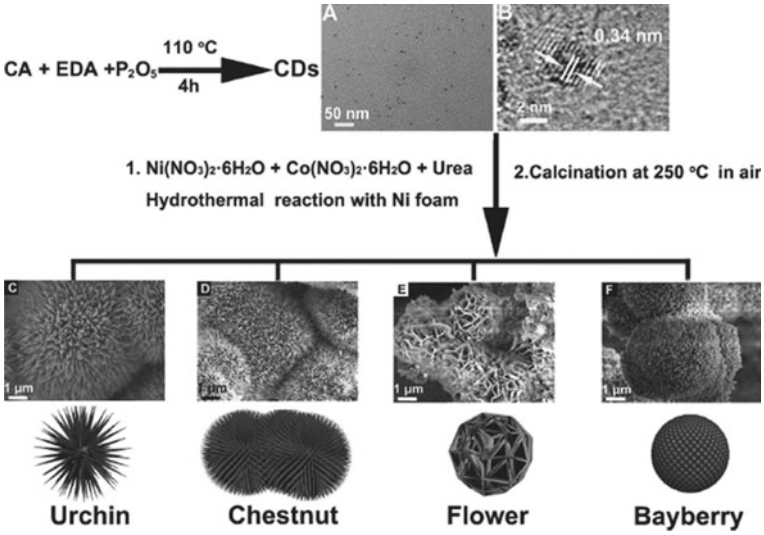

Fig. 2 **a**, **b** the hydrothermally synthesized CQDs and **c**–**f** CQDs/$NiCo_2O_4$ composite with different morphologies [22]. Copyright 2016, Wiley

in a N-CQDs/MnO_2 composite. In addition, it roughens the surface with enhanced conductivity and improved OER activity [21]. The same strategy was used by Xiong et al. where the morphology of $NiCo_2O_4$ was controlled by adjusting inventory rating of CQDs-from sea urchin to flower, and bayberry, as displays in figure [22] (Fig. 2).

Moreover, few reports claimed to enhance the electrochemical activity by employing the CQDs for enhancing the conductivity of overall composite. The use of CQDs effectively eliminates the Schottky barrier from the catalyst-electrolyte interface, and catalyst-electrode interface [23]. For instance, Kang et al. has reported CQDs/SnO_2–Co_3O_4 composite for electrocatalytic water oxidation where CQDs enhanced the conductivity of composite by facilitating the enhanced electron transfer between the surface and active sites [24]. In another report, Yang et al. reported the synthesis of Co, Fe co-doped CQDs where CQDs enhanced the electrocatalytic OER activity and stability by accelerating the electron transfer [25].

The remarkable performances of CQDs-based composites manifested themselves as a perfect fit for various electrocatalytic applications. Nevertheless, CQDs-based composites also show enhanced photocatalytic activities for various applications such as H_2 production, CO_2 reduction, and organic contamination degradation. For instance, Sargin et al. reported CQDs/TiO_2/Pt catalyst for H_2 production, where, up on the absorption of the photon, the sp^2-conjugated CQDs produce excited electrons in the valence-conduction-band bridge [26]. Thereafter, the electrons get transfer to the conduction band of TiO_2 which results in the production of H_2—the report claimed to produce 1458 μ mol g^{-1} of H_2 after 2 h (Fig. 3).

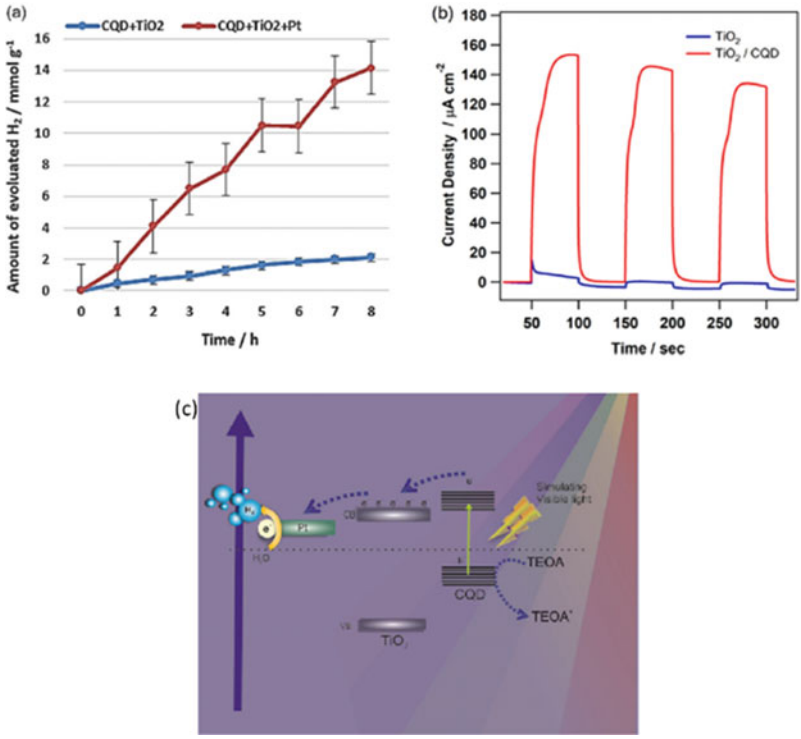

Fig. 3 **a** The photocatalytic hydrogen evolution obtained by gas chromatography, **b** the photocurrent density (using chronoamperometry method) of TiO_2, and CQD/TiO_2, and **c** Schematic representation of photocatalytic hydrogen evolution mechanism of CQD/TiO_2/Pt [26]. Copyright 2019, Elsevier

In addition, Gogoi et al. developed CQDs/CdS composite for UV-Vis driven photocatalytic water-splitting reaction-the use of CQDs enhanced the performance of catalyst by 50% as compared to bare CdS [27]. Moreover, Kulandaivalu et al. synthesized CQDs/Cu_2O nanocomposite for the photoreduction of CO_2. It is reported that CQDs improved the overall process by 54% as compared to bare Cu_2O. The mechanism of photoreduction of CO_2–C_2H_6 is reported as follows-Upon visible light-driven photoexcitation of Cu_2O, the electron-hole pairs generate in the conduction band (CB) and valence band (VB) of Cu_2O respectively. The redox potential of pure Cu_2O was reported -1.0 V and $+1.1$ V for VB and CB respectively which makes it competent to reduce CO_2–C_2H_6 ($E^o = -0.27$ V). The process began as the electrons in the CB of Cu_2O interacts with CO_2 to produce CO_2^- radicals, simultaneously, the holes in the VB of Cu_2O reacted with H_2O to form OH and H_2 which further oxidized into O_2 and H^+. The H radicals formed as the electrons reacted with H^+ ions which then reduce the CO_2^- radicals to form CH_3 which further dimerized to generate C_2H_6. However, in this process, the bare Cu_2O displayed poor efficiency because of rapid recombination rate of photogenerated electron and hole pairs in the

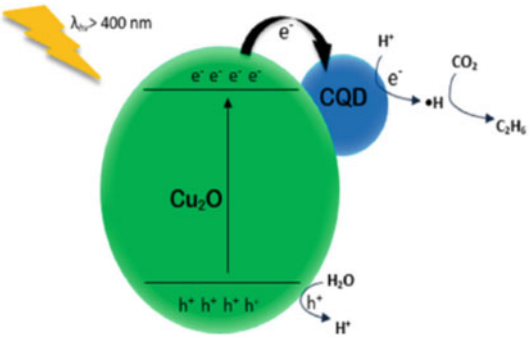

Fig. 4 Schematic illustration of the mechanism of photocatalytic reduction of CO_2 over CQD/Cu_2O composite [28]. Copyright 2018, Elsevier

CB and VB of Cu_2O. This obstacle was cleared out by making a composite CQDs/Cu_2O, where CQDs assisted in the photostability of composite, and preventing the recombination of photogenerated charge carriers [28] (Fig. 4).

Using same approach, CQDs-based composites were also exploited in photodegradation of organic pollutants. For example, Tyagi et al. immobilized water soluble CQDs over electro-spun TiO_2 nanofibers [29]. This composite was used for the photodegradation of methylene blue dye. In this report, CQDs played an important role to separate the photo-excited charge carriers in TiO_2 under the exposure. The production of O_2 (due to the electrons transferred from CB of TiO_2 to CQDs) and OH radicals (due to holes in the VB of TiO_2) at the surface of composite resulted the degradation of organic pollutant 2.5 times faster than only TiO_2 nanofibers. In another report, Ramar et al. compared the performance of undoped-CQDs and nitrogen-doped CQDs for the degradation of MB dye under the exposure of solar light [30].

3 Semiconductor QDs (SQDs)-Based Composites

Having the size comparable to three-dimensional exciton Bohr radius, the movement of internal electrons get restricted in QDs [31]. Here, in case of the semiconductors, if the size of nanomaterials gets smaller or equal to its exciton Bohr radius, then, the internal energy of that material converts from nearly continuous energy band (in bulk materials) to discrete energy levels. Therefore, the bandgap of semiconductor QDs (SQDs) can be tuned by modulating its size. This makes SQDs a highly efficient class of materials for the applications in sensing, imaging, medicines, and displays. Among them, SQDs-based catalysis, prominently in the field of photocatalysis- for hydrogen production, CO_2 reduction, and organic pollutants degradation, will be discussed later in this section.

In general, when the light energy ($h\nu$) equal or greater than the bandgap energy (E_g) of the SQDs incident on the SQDs, compel electrons of the VB (of SQDs) transit to the CB (of SQDs). Upon excitation of electrons to the CB, the holes remain in the

VB of SQDs, this process is called electron-hole pair (EHP) generation. These photo-generated EHPs later contribute in the catalytic reactions-electrons are reductive in nature which are necessary in hydrogen production and CO_2 reduction, whereas, the holes are oxidative in nature which required in organic pollutants degradations [32]. Typically, SQDs-driven photocatalytic reactions face several challenges, including the surface traps of EHPs, the active sites blockage by reaction intermediates or agglomerates, and rapid recombination of EHPs. Therefore, to make this process more efficient, a co-catalyst generally introduced with SQDs. This SQDs-based composite work in synergy to separate EHPs, and avail them for respective catalytic reactions [33].

Chalcogenides (MX) QDs (ChQDs) are the most exploited class of QDs for diverse range of applications, where M represent the transition metals, and X signify the chalcogens (S, Se, and Te). The VB and CB of ChQDs are mainly formed because of the chalcogen, and transition metal element respectively. In general, ChQDs have the characteristics of narrow and direct bandgap which gives them advantage of rapid EHP generation. But, the maximum of generated EHPs quickly recombine. In addition, ChQDs also have the tendency to agglomerate during use, and due to the long exposure of light, the photogenerated holes get accumulate on the surface, resulting in the photo-corrosion. However, there are various strategies which can solve this issue with the advantage of overall enhancement in the catalytic efficiency. For instance, Liang's group fabricated the composite of CdS QDs with graphitic carbon nitride (g-C_3N_4) [34]. In this report, phosphorus modified g-C_3N_4 tubes were used as a porous matrix with CdS QDs which could protect QDs from photo-corrosion and agglomeration. These strategies promote the EHPs separation, suppress radiative recombination, extend the lifetime of carriers, and promote the reusability of catalyst (Fig. 5).

In another report, as templates of CdS QDs, mesoporous silica/halloysite nanotubes (HNTs) was investigated [35]. This composite was employed for the production of H_2 gas, the production of H_2 could reach at 2600 μ mol h^{-1} g^{-1}, with the reusability of five cycles. Moreover, to achieve the H_2 production rate of 13129 μ mol h^{-1} g^{-1}, Zhuge et al. fabricated MoS_2 coated CdS QDs [36]. The encapsulated MoS_2 on the surface of CdS QDs promote internal charge separation, and prevent photo-corrosion (Fig. 6).

In other reports, it's been studied that ZnS shell could prevent the QDs from photo-corrosion. ZnS shell inhibited the formation of trapping state on the surface of QDs, and facilitated the transfer of photogenerated carriers from QD core to the surface [37, 38]. Furthermore, the photocatalytic activity of ChQDs-based cocatalysts can be improved by surface modification including surface stoichiometry manipulation, construction of core/shell structure, doping with inorganic ions, ligand removal and exchange [39]. The surface ligands not only protect QDs from aggregation but they also regulate the growth rate of ChQDs, depending upon its chain lengths [40]. In addition, Bajorowicz et al. reported that the thioglycolic acid (TGA) caped CdTe QDs showed better photocatalytic performance than the mercaptopropionic acid

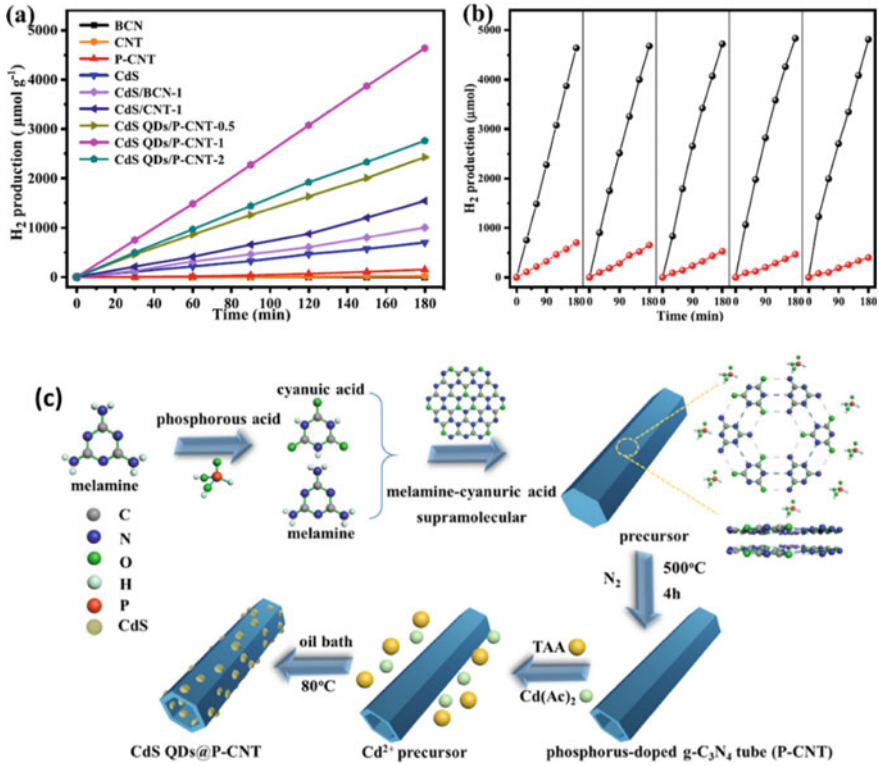

Fig. 5 **a** Photocatalytic H_2 production rates of the BCN, CNT, P-CNT, CdS, CdS/CNT, and CdS QDs/P-CNT, **b** stability tests for photocatalytic H_2 evolution, and **c** schematic illustration of CQD/g-C_3N_4 composite [34]. Copyright 2020, Elsevier

(MPA) capped CdTe QDs. TGA, being a shorter ligand as compared to MPA, minimizes the transfer distance of photogenerated electrons [41]. To improve the separation of EHPs, a suitable ligand induces intimate contact between the QDs and the semiconductor substrates. In this regard, Sakizadeh et al. found that the L-cysteine capped CdS QDs and reduced graphene oxide (rGO) don't integrate with each other because both have negative charge on the surface. Whereas, by exchanging the L-cysteine to cysteamine, the QDs became positively charged which interacted well with rGO through electrostatic attraction [42]. The use of organic ligands doesn't always improve the photocatalytic performance of ChQDs-based composites. Sometimes, it creates the physical or electronic barriers for photogenerated charge carriers from QDs to cocatalysts [43] (Fig. 7).

Along with, the surface stoichiometric ratio of M and X (in MX ChQDs) also manipulates the photocatalytic performance of ChQDs. For instance, Huang et al. synthesized Se rich CdSe QDs, these extra Se sites acted as hole traps which prohibit the interfacial charge transfer from CdSe QDs to the sacrificial agents, resulting

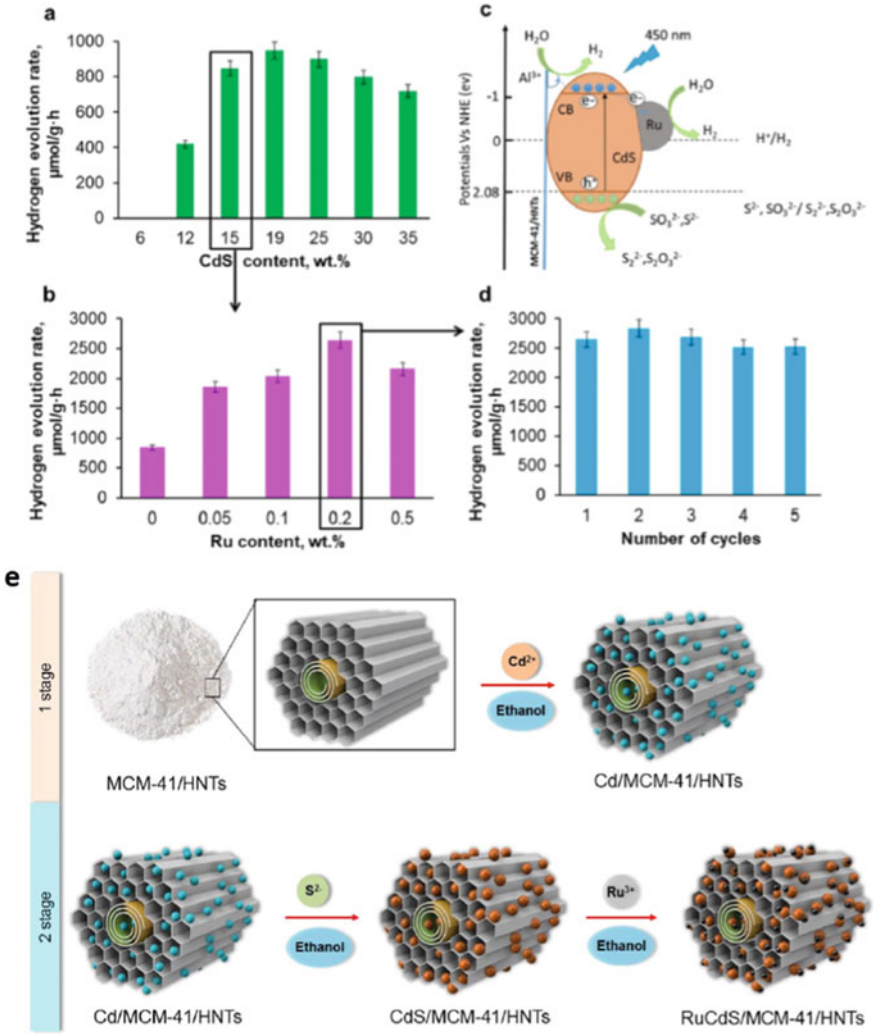

Fig. 6 Photocatalytic activity of **a** CdS and **b** $Ru_{15}CdS$ QDs stabilized on the surface of MCM-41/HNT (depending on CdS and Ru content), **c** schematic representation of the photocatalytic mechanism under visible light illumination, **d** stability of $0.2Ru_{15}CdS$/MCM-41/HNT composite for five cycles of H_2 evolution, and **e** schematic of fabrication steps of RuCdS/MCM-41/HNT composite [35]. Copyright 2021, American Chemical Society

the enhancement in EHPs recombination which leads to poor photocatalytic performance, in the contrary, the Cd-rich CdSe QDs, surface Cd atoms acted as active sites, resulting in the enhancement in photocatalytic performance [44] (Fig. 8).

The ionic doping/modification (cationic or anionic) in ChQDs also an effective approach for enhancing the photocatalytic performance. For instance, to prevent the

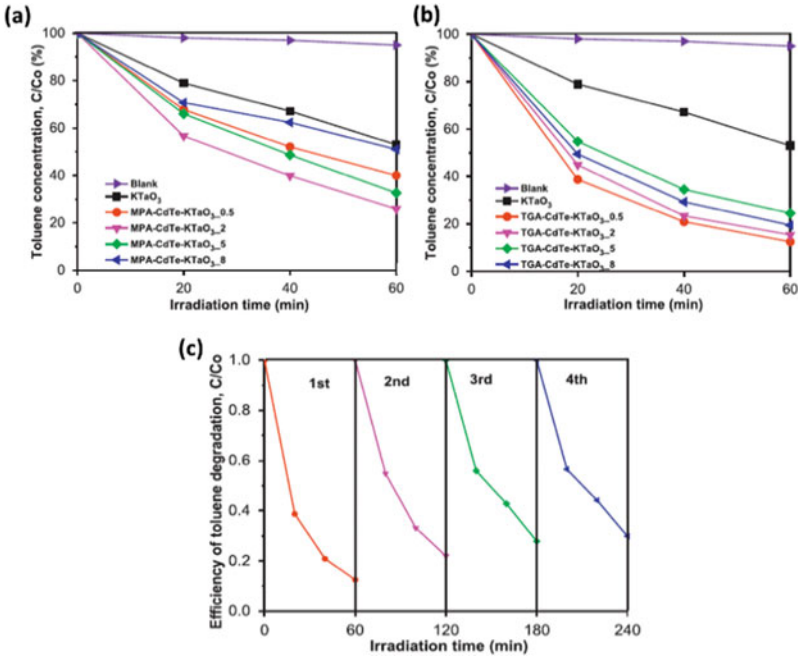

Fig. 7 Efficiency of **a** MPA-CdTe-$KTaO_3$, and **b** TGA-CdTe-$KTaO_3$ for the photodegradation of toluene, and **c** recycling performance of TGA-CdTe-$KTaO_3$-0.5 composite toward toluene photodegradation under LED light illumination ($\lambda_{max} = 415$ nm) [41]. Copyright 2016, Elsevier

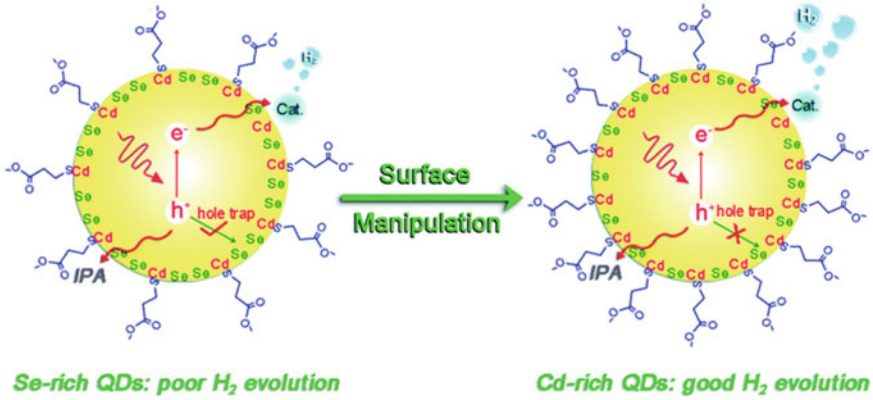

Fig. 8 Hydrogen evolution in Se-rich and Cd-rich CdSe QDs [44]. Copyright 2018, Royal Society of Chemistry

EHPs recombination, Pu et al. synthesized Mn^{2+} doped ZnSe QDs [45]. This report explored that the photoluminescence (PL) decay (from 50 to 1000 μs) of the crystal can be adjusted by varying the doping concentration. In another report, S^{2-} modified QDs show low dielectric barrier with its cocatalyst, and facilitate the transfer of photogenerated charge carriers.

In general, the structure of the cocatalyst of QDs also affect the photocatalytic performance of overall composite. For example, Ma et al. fabricated a nanocomposite of 0D CdS QDs and 2D ZnO nanosheets [46]. The direct Z-scheme heterojunction promoted the separation of photogenerated EHPs. Recently, the researchers have also explored the photocatalytic performance of ChQDs coupled with metal-organic frameworks (MOFs), and covalent organic frameworks (COFs) [47, 48]. The MOFs and their derivatives offer high surface area, and high porosity with adjustable pore size which is in favor for effective photocatalytic reaction. However, the wide bandgap of MOFs limits its use in visible-light-driven photocatalysis [49–51]. Thus, the coupling of MOFs with ChQDs facilitate their use in photocatalytic reactions. For example, Mao et al. used SILAR approach for the integration of CdS QDs into thiol-laced UiO-66 (UiOS) for photocatalytic H_2 evolution [52]. Similarly, COFs also have advantages of tunable structure, good thermal, and chemical stability, large surface area, and high charge carrier mobility [53]. However, COFs are organic porous crystalline materials, composed of covalent-bonded nonmetallic elements (such as C, N, O, B etc.) [54]. Huang et al. designed a composite composed of CdS QDs and covalent triazine-based frameworks (CTF-1) [48]. The prepared composite showed the enhanced photocatalytic H_2 evolution by the factor of 4 and 55 times as compared to bulk CdS and pristine CTFs, respectively (Fig. 9).

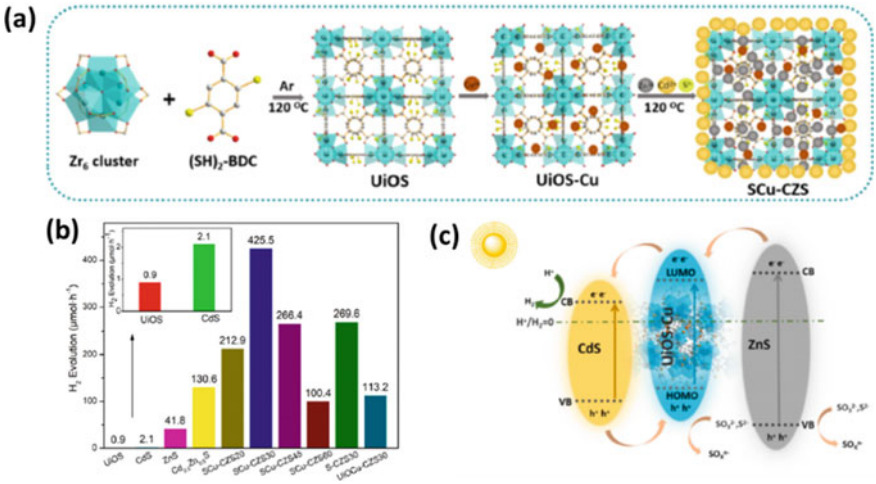

Fig. 9 **a** Schematic illustration of synthesis procedure of SCu-CZS composite, **b** the photocatalytic H_2 evolution performance, and **c** schematic illustration of interfacial charge transfer process in SCu-CZS catalyst [47]. Copyright 2020, Elsevier

4 Perovskite QDs (PQDs)-Based Composites

Perovskites QDs (PQDs), a unique class of photocatalysts, are promising in the field because of its structural simplicity and pliability, photo-absorption tunability, long diffusion lengths, prolonged lifetime and high mobility of charge carriers [55]. Having the chemical formula of ABX_3, these perovskites are most exploited in the field of photocatalysis, where A and B are cations, and X is an anion. The cation A can be an organic (methylammonium, formamidinium) or inorganic (Cs^+), the cation B represents a divalent metal ion (such as Pb, Sn, Bi, etc.), and X is the halide anion (Br, Cl, or I). By modulating the composition of cation/anion, the photo-absorption of PQDs can be enabled from UV to NIR region [56]. Furthermore, the size of PQDs play a significant role in dictating its photocatalytic performance. For instance, Hou et al. employed four different sizes (3.8, 6.1, 8.5, and 11.6 nm) of $CsPbBr_3$ PQDs (dispersed in ethyl acetate/water) for the CO_2 reduction application. To measure their comparative performance towards CO_2 reduction, as-mentioned PQDs were illuminated under solar exposure for 8 h individually. It was found that the PQDs of size 8.5 nm show better performance [57] (Fig. 10).

In addition, the crystalline phase of PQDs also elucidate its performance- Guo et al. reported that the PQDs of crystalline phase are more active than the orthorhombic phase $CsPbBr_3$ PQDs for their application in CO_2 reduction [58]. Moreover, there are several other strategies which enhances the performance of PQDs-based hybrids for their application in CO_2 reduction. For instance, Mu et al. reported cobalt-doped $CsPbBR_3/Cs_4PbBr_6$ QDs/hexafluorobutyl methacrylate [59]. To elaborate on this, Wu et al. investigated the visible light-driven CO_2 conversion performance by using the $MAPbI_3$/Fe-porphyrin-based MOFs composite [52]. Choosing MOFs for making hybrid with PQDs gives several advantages such as longevity of PQDs, improving charge transfer, and prevent agglomeration [60–62]. In several reports, the silica and organic layers were used as the protecting layer of PQDs [63–67]. However, the flow of charge gets hampered by using these layers. Alternatively, the use of conductive protecting layer to wrap PQDs gives several advantages including better electron transfer, enhances the durability and intact the morphology of PQDs [68]. Along with, the researchers also exploited PQDs for the photodegradation of organic contaminants. For example, Gao et al. employed $CsPbX_3$ for the degradation of methyl orange (MO). This report shows that the photocatalytic activity increases with the concentration of catalyst up to certain quantity, but beyond that limit, the photocatalytic activity tends to decrease [69]. In another report, Qian et al. studied the photocatalytic degradation of tetracycline chloride (TC-HCl) using $CsPbBr_3$ as a photocatalyst [70]. Recently, PQDs has also been employed as a visible light-driven photocatalyst for the stereoselective C–C oxidative coupling of α- aryl ketonitriles [71]. In another report, the surface tuning of PQDs was achieved using zwitter ions- $CsPbBr_3$ PQDs were capped by zwitterionic ligand (3-(N,N-dimethyloctadecylammonio)propanesulfonate, DMOA-PS) [72]. Being a diverse photocatalyst, PQDs-based hybrids also found their application in NO gas

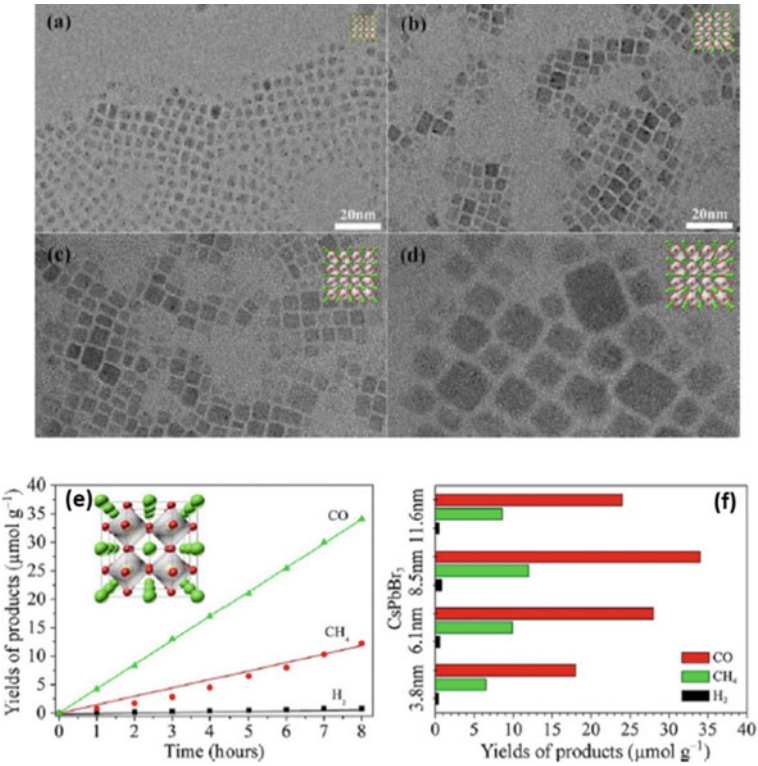

Fig. 10 TEM images of **a** 3.8, **b** 6.1, **c** 8.5, and **d** 11.6 nm $CsPbBr_3$ QDs, solar light-driven (300 W Xe lamp) CO_2 reduction, **e** 8.5 nm $CsPbBr_3$ QDs, and **f** for different sizes of $CsPbBr_3$ QDs [57]. Copyright 2017, Wiley

oxidation. For example, Huo et al. used $FAPbBr_3$/A-SiO_2 composite for NO gas degradation [72].

5 Conclusions

This chapter illustrated the role of CQDs-, SQDs-, and PQDs-based composites for their application in catalysis. As-mentioned researches promise the great potential of QDs based composites for the catalysis related applications. The range of electron hole pair (EHP) generation is critical in developing an efficient photocatalytic application. The studies promoted applications such as dye degradation, organic pollutant degradation, CO_2 reduction, hydrogen evolution reaction (HER), oxygen evolution reaction (OER) activity. However, rigorous research is still required to exploit the full potential of these composites.

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Quantum Dots Nanocomposites Bioimaging Probes

Seyed Ahmad Dehdast and Omid Pourdakan

Abstract Quantum dots nanocomposites (QDNCs) are highly fascinating nanomaterials for bioimaging application due to their distinctive optical properties. By using the unique optical properties of quantum dots based nanocomposites, the performance of biomedical imaging can be significantly improved in terms of their contrast, resolution, sensitivity, specificity, and penetration depth. For this reason, recently, the use of QDNCs in bioimaging and medical imaging studies has received much attention and is increasingly being used. Recent QDNCs as a bioimaging probes were used in fluorescent imaging, positron emission tomography (PET imaging) and magnetic resonance imaging (MRI) investigations. In this chapter different types of QDNCs, their properties, advantages, and emerging applications of these nanomaterials in ex-vivo and preclinical study summarized and presented.

Keywords Quantum dots nanocomposites (QDNCs) · Bioimaging probe · Fluorescence imaging · PET imaging · Magnetic resonance imaging (MRI)

List of Abbreviations

A-GQD	Adenine-modified Graphene Quantum Dots
Ag_2Se	Silver Selenide
AKI	Acute Kidney Injury
AOM	Azoxymethane
BP	Black Phosphorus
BPQD	Black Phosphorous quantum dots
C18-PMH	Poly(maleic anhydride-alt-1-octadecene)
Cd	Cadmium
CdS	Cadmium Sulfide
CdSe	Cadmium Selenide

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CIS (CuInS)	Copper Indium Sulfide
CKD	Chronic Kidney Disease
CQDNCs	Carbon Quantum Dots based nanocomposites
CQD-WS	Carbon Quantum Dot-tungsten sulfide
CRET	Cerenkov resonance energy transfer
CT	Computed Tomography
DAPI	4′,6-Diamidino-2-phenylindole
DMF	Dimethylformamide
DSS	Dextran Sodium Sulfate
EDTA	Ethylenediaminetetraacetic acid
FA	Folic Acid
FL	Fluorescence
FLIM	Fluorescence Lifetime Imaging
FR	Folate Receptor
FRET	Förster Resonance Energy Transfer
Gd-DTPA	Gadolinium-diethylenetriaminepentaacetic acid
Gd-DOTA	Gadolinium −2,2′,2″,2‴-(1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrayl)tetraacetic acid
GQDNCs	Graphene quantum dots based nanocomposites
GSH	Glutathione
ICG	Indocyanine Green
In	Indium
InP	Indium Phosphate
InS	Indium Sulfide
LED	Light-emitting diode
MB	Methylene Blue
MMP	Matrix metalloprotease
MoS_2	Molybdenum Disulfide
MR	Magnetic Resonance
MRI	Magnetic Resonance Imaging
MTT	2,5-Diphenyl-2H-tetrazolium bromide
N-GQD	Nitrogen doped-Graphene Quantum Dots
NIR-I	Near-Infrared I
NIR-II	Near-Infrared II
OCT	Optical Coherence Tomography
OPFI	One-photon Fluorescence Imaging
PA	PhotoAcoustic
PbS	Lead Sulfide
PEG	Polyethylene glycol
PET	Positron Emission Tomography
PET	Photoexcited Electron Transfer
QCE	Quantum Confinement Effect
QD	Quantum Dots
QDNC	Quantum dots nanocomposites
QY	Quantum Yield

RGD	Arginine-Glycine-Aspartic Acid
RP	Red-Phosphorus
RQDNCs	Radioactive Quantum Dots nanocomposites
TMDs	Transition metal dichalcogenides
TPFI	Two-photon Fluorescence Imaging
WS_2	Tungsten Disulfide
WP	White Phosphorus
ZnS	Zinc Sulfide
ZnSe	Zinc Selenide

1 Introduction

Quantum dots nanocomposites (QDNCs) are a multipurpose material that can be employed in a broad range of applications. This is due to special physical and chemical traits of QDNCs. Their surface chemistry can be easily manipulated and changed. Because of these unique properties, quantum dots are one of the attractive candidate used nanomaterial in research and medicine with high potential for use in many medical devices and industrial products. Due to the small size of quantum dots (less than 20 nm), they are ideal for imaging applications and drug delivery to various tissues. Also, QDNCs have proved to be efficient catalysis material. These properties make that's applicable in medicine [1]. Moreover, QDNCs have the unique optical attributes, practical and fantastic physical capacity to specific wavelengths of light for use in bioimaging. Recently, have been discovered quantum dots can be useful in various scientific and industrial disciplines such as medical engineering, imaging, electronic devices and solar cells. These nanostructures have also been employed to develop light-emitting diodes' quantum and electroluminescence efficiency. Providing a wide range of colors and optical coding in many colors for biological experiments is one the most important their characteristics [2]. Anyway, according to the mentioned features, quantum dots possess a variety of features that make them great nanobiotechnology instruments. These nanomaterials are useful for imaging because of high capacity for chemical functionalization and the wide variety of surface functions and bioconjugation that may be attached to the quantum dots and their exceptional chemo-physical characteristics. Modification of QDNCs makes that's useful highly sensitive and selective for diagnostic and theranostic systems. QDNCs have high therapeutic potential, for the reason of high surface loading of medication, genes and their controllable release of the loaded materials. Collectively, quantum dots are very fantastic and adaptable nanomaterial for future medical engineering, bioengineering and nanomedicine uses [3]. In this chapter, the potential and recent research applications of quantum dots nanocomposites for imaging in medicine and biology are explored.

2 Bioimaging

Bioimaging is a scientific method that enables the real-time perception of biological processes in body, with the use of non-invasive tools. Its purpose is to cause negligible interference to live processes and provide information on the 3D structure of the observed construction. Bioimaging technologies are used in various fields such as fundamental and medical sciences for anatomical and physiological examining, and in gathering research information [4].

Bioimaging has numerous applications in various fields, including medicine, biology, chemistry, materials science, and environmental science. In medicine, bioimaging is used for diagnosis, treatment planning, and monitoring of diseases. For example, X-ray imaging is commonly used to detect fractures and tumors, while magnetic resonance imaging (MRI) is used to visualize soft tissues and organs. In biology, bioimaging is used to study cellular processes, such as mitosis and apoptosis, and to investigate the structure and function of biomolecules, such as proteins and nucleic acids. In chemistry, bioimaging is used to study chemical reactions and to develop new materials, while in environmental science, bioimaging is used to study the distribution and transport of pollutants in the environment [5].

Complex bioimaging applications require multi-modal and multi-scale imaging techniques that can involve ultrasound and light imaging to comprehend complicated structures and interactions profound inside the body [6]. Many clinical imaging techniques such as ultrasound imaging (sonography), X-ray computed tomography (X-ray CT), optical coherence tomography (OCT), and magnetic resonance imaging (MRI) utilize the entire energy spectrum for imaging. Furthermore, research methods include electron microscopy, mass spectrometry imaging, fluorescence tomography, biochemical luminescence, various forms of OCT, and optoacoustic imaging. Light microscopy methods include confocal, multi-photon, total internal reflection, and super-resolution fluorescence microscopy employ the whole energy spectrum for analysis [7].

3 Quantum Dots Nanocomposites as Fluorescence Imaging Probe

Biocompatible QDNCs are typically composed of a nanoscale core made of semiconductor materials such as CdSe or InP, which emit light when excited by an external energy source such as a laser. The core is then coated by a semiconductor substance for example ZnS or ZnSe, which serves to passivate the surface of the core and improve its photostability.

In addition, the outer layer of biocompatible QDNCs is often modified with a coating of silica, which can provide a high surface area for further modifications with biomolecules including nucleic acids, antibodies, peptides, and small-molecule

ligands. These modifications can enable the QDs to target specific molecules on the cancer cells surface, allowing for highly selective imaging and even therapy [8].

Dong et al. successfully prepared Ag_2Se quantum dots (QDs) using a solvothermal method, which emitted light at a wavelength of 1300 nm in the second near-infrared (NIR-II) window. The Ag_2Se QDs were then modified to enhance their properties such as water solubility, colloidal stability, photostability, and biocompatibility. These modified Ag_2Se QDNCs were used for deep imaging of arteries and organs in vivo, providing higher spatial resolution than indocyanine green (ICG), as a near-infrared (NIR-I) fluorescent substance that using in medical diagnosis. Investigation of in vitro cell toxicity were conducted using PEGylated poly (maleic anhydride-alt-1-octadecene) Ag_2Se (C18-PMH-PEG-Ag_2Se) QDNCs on L929 cell line, and the results showed that the cells remained viable with different concentrations of the QDNCs, without any significant changes in their viability. In vivo experiments involved administering C18-PMH-PEG-Ag2Se QDNCs via tail intravenous injection, resulting in high spatial resolution images of vascular structures and deep organs within the body, with a resolution of approximately 100 μm. The minimal autofluorescence, low scattering, and absorption of the NIR-II emission at 1300 nm enabled highest penetration depth for deep organ imaging with high resolution, making it possible to image organs like the liver and spleen [9].

The utilization of QDNCs in clinical applications has been constrained due to concerns regarding their potential toxicity, particularly with regards to their core material, cadmium. Yaghini et al. have developed a novel type of QDNCs that are free of heavy metals and cadmium (Cd), and are biocompatible, relying on indium (In) as the core material. These QDNCs have good photoluminescence quantum yield (QY) and have been evaluated for lymph node mapping using fluorescence lifetime imaging (FLIM) (Fig. 1). In vitro toxicity studies were conducted on MCF-7 cells using MTT assays, and No notable cell viability reduction was observed in QDNCs treated cells. Subsequent ex vivo imaging of regional lymph nodes following subcutaneous injection through the paw of rats demonstrated the potential of these QDNCs in the context of lymph node mapping. Upon injection, the QDNCs exhibited a preferential accumulation in two specific lymph nodes that drain the thoracic mammary fat pads in rats. The investigation on biodistribution, conducted through chemical extraction, revealed that the retention of the QDNCs is highly limited to these regional lymph nodes, with only negligible amounts detected in other organs. Remarkably, the QDNCs promptly localized within minutes of injection, and their photoluminescent properties remained stable for a prolonged duration. In contrast, blue dyes tend to swiftly drain from lymph nodes. This protracted retention of the QDNCs could prove advantageous in a clinical context, as it has the potential to prevent false positive outcomes associated with the rapid migration of dyes through the lymphatic channel, all while being logistically more manageable [10].

Jeong et al. have developed a protease-activatable near-infrared II (NIR-II) quantum dot nanocomposites (QDNCs) probe that was sensitive to matrix metalloprotease (MMP) activity. The high performance luminescent PbS/CdS/ZnS multi-shell NIR QDNCs with protease-activatable (PA) modulators (AcM) linked to probe

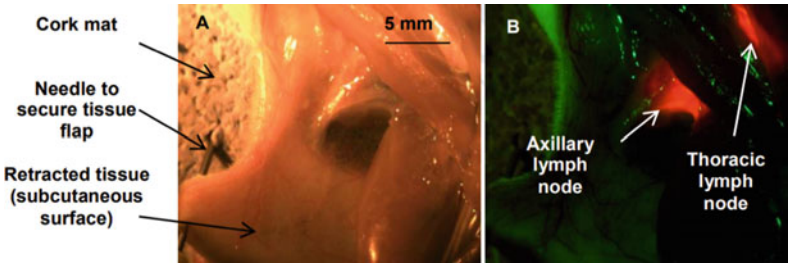

Fig. 1 **a** The image on the left side displays color, while **b** the right part exhibits photoluminescence. This photoluminescence image showcases the armpit area after the QDNCs administration. In order to illuminate the tissues, a collimated LED lamp with a peak emission at 455 nm was employed. The green emission observed in image, **b** is indicative of tissue autofluorescence, while the red emission corresponds to the photoluminescence of the QDNCs in the axillary and thoracic lymph nodes. Reproduced with permission [10]. Copyright 2016, Elsevier

surface. The photoexcited electron transfer (PET) depends on QDNCs and the methylene blue (MB) quencher distance and has an effect on fluorescence (FL) intensity which can be modulated by AcM. The modulation mechanism can be applied to NIR-II wavelength and longer and is different from FRET mechanism however it is not fully discovered yet. Signal amplification was observed at colorectal tumor site by ex-vivo fluorescent imaging and deep imaging potential was approved by phantom experiments. PET-mediated NIR-II QDNCs can be extended to other protease probes, enabling high-resolution, real-time, noninvasive FL imaging (Fig. 2) [11].

Carbon quantum dots based nanocomposites (CQDNCs) are a novel class of carbon nanomaterials that have gained growing interest in recent times owing to their distinct physical and chemical characteristics. These nanomaterials are generally characterized by their sub-10 nm dimensions, exhibiting a spherical morphology accompanied by a crystal lattice configuration and surface-bound chemical moieties that play a pivotal role in their luminescent features [12].

One of the most important features of CQDNCs is the quantum confinement effect (QCE), which occurs when the size of the CQDNCs is smaller than its exciton Bohr radius. This effect leads to discrete energy levels within the CQDNCs, which in turn results in tunable emission wavelengths and high quantum yields. Furthermore, CQDNCs are less cytotoxic and environmentally hazardous than traditional semiconductor quantum dots, making them a more attractive option for biomedical and environmental applications [13, 14].

In addition, CQDNCs have suitable water solubility, chemical stability, and photobleaching resistance, as well as ease of surface functionalization and large-scale preparation. These properties make them an attractive candidate for various applications, such as bioimaging, drug delivery, and optoelectronics [14].

Graphene quantum dots based nanocomposites (GQDNCs), on the other hand, are derived from graphene, a two-dimensional carbon material that has unique electronic and mechanical properties. GQDNCs are typically smaller than 10 nm in size and have a tunable bandgap, making them attractive for electronic and optoelectronic

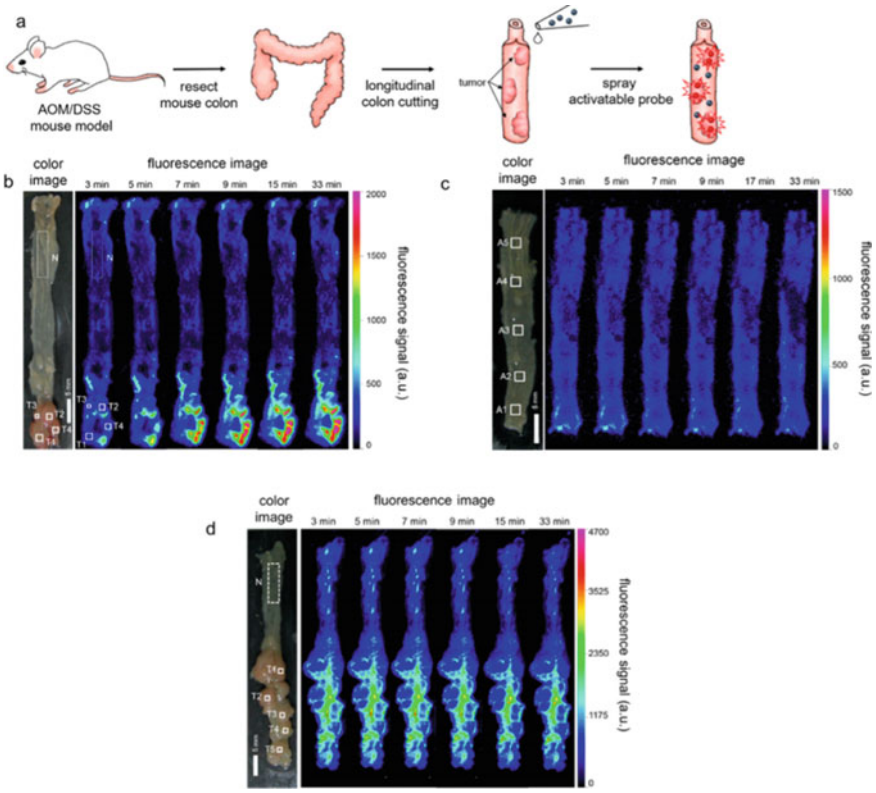

Fig. 2 **a** The diagram illustrates the process of ex vivo imaging of colon cancer in mice using near-infrared II fluorescent (NIR-II FL) technology by applying an activatable probe. At first, colon tissue is extracted from a mouse with a tumor treated with Azoxymethane/dextran sodium sulfate (AOM/DSS). The tissue is then cut longitudinally, and an even distribution of the PA-NIR QDNCs probe is sprayed onto the surface of the tissue. **b** Observe the results of the ex vivo FL imaging of colon cancer by applying the protease-activatable probe, PA-NIR QDNCs. The color image showcases the intact model of mouse colon cancer, while the time-dependent FL image demonstrates the changes that occur after the spraying of the probe. The designated tumor regions, T1–T4, and the normal colon tissue, N, are identified. **c** Similarly, examination of the ex vivo FL imaging of a healthy colon by employing the same spraying technique with PA-NIR QDNCs. The color image illustrates the intact model of the mouse colon, and the time-dependent FL image reveals the alterations that transpire following the application of the probe. Once again, the tumor regions, T1–T4, and the normal colon tissue, N, are explicitly labeled. **d** Investigation of colon cancer's ex vivo FL imaging using NIRQDs without the activatable probe. The color image presents the model of mouse colon cancer in its entirety, whereas the time-dependent FL image depicts the changes that arise after the spraying of the probe. Reproduced with permission [11]. Copyright 2017, American Chemical Society

applications. They also have high surface area and good biocompatibility, which make them promising candidates for biomedical applications such as drug delivery and bioimaging [15].

Using carbon soot as precursor and treating it with nitric acid and then functionalized with Polyethylene glycol (PEG)1500, Yang aim of this study was to compare the properties of synthesized CQDNCs to commercially available CdSe/ZnS quantum dots (QD) in terms of imaging and cytotoxicity. CQDs-PEG and QDs were excited with femtosecond pulsed laser excitation at 800–880 nm and revealed that CQDs-PEG had higher biocompatibility and luminescence, with a quantum yield of 20%, and smaller size compared to CdSe/ZnS QDs. Cytotoxicity in vitro and in vivo test was performed on human breast cancer MCF-7 and human colorectal adenocarcinoma HT-29 cell line and CD-1 mice respectively and showed no significant effect of CQDNCs compare to QD [16].

A one-pot hydrothermal synthesis with monosaccharides in Wheat straw and bamboo residues as precursor have been utilized to produce CQDs for in vitro and in vivo imaging. The resultant CQDs that range from 2 to 6 nm showed approximately 13% QY. In a comparison to the commercially available fluorophore 4′,6-diamidino-2-phenylindole (DAPI), in a different range temperature from 4 to 60 °C and photobleaching treatment, Huang et al. CQDs exhibit stable fluorescence properties. L929 fibroblasts cell line was used to investigate cytotoxicity and showed cell viability more than 90% in highest concentration. For in vivo imaging application a nude mouse with Smmc-7721 tumor was injected through tail's vein and mouse organs were imaged from 0 to 24 h. CQDs showed low cytotoxicity, antioxidant activity and good feature for imaging application [17] (Fig. 3).

Liu et al. synthesized graphene quantum dots doped with nitrogen (N-GQD) by solvothermal method and using Dimethylformamide (DMF) as solvent and nitrogen source, which had a size of 3 nm and excitation in the region of 390 nm, and were used for two-photon and deep tissue imaging. To investigate the ability of two-photon imaging, the nanoparticles were incubated with HeLa cells and excited by a femtosecond laser with a wavelength of 800 nm, which showed high fluorescent contrast with green color around the nuclei of the cells and the ability to label the membrane and cytoplasm of HeLa cells. They showed no penetration into the nucleus. Cell viability studies in different doses showed that these nanoparticles are not toxic. In order to evaluate the deep tissue imaging of GQD, a tissue phantom was used and the obtained results show that the nanoparticles penetrate deeply. The large imaging penetration depth of 1800 μm achieved by N-GQD in tissue phantoms significantly extends the fundamental imaging depth limit of two-photon microscopy (Fig. 4) [18].

Lou et al. synthesized adenine-modified graphene quantum dots (A-GQDs) with microwave-assisted method. The A-GQDs showed 3–5 nm lateral size, single-few-layer thickness, 21.63% quantum yield at 350 nm excitement wavelength and 4.47 ns average lifetime. Duo it's biocompatibility and two photon green fluorescence production is suitable for two-photon fluorescent cell imaging. The novel white light-activated antibacterial properties of the A-GQDs was observed for the first time. With their exceptional photoluminescent performance and facile biological conjugation,

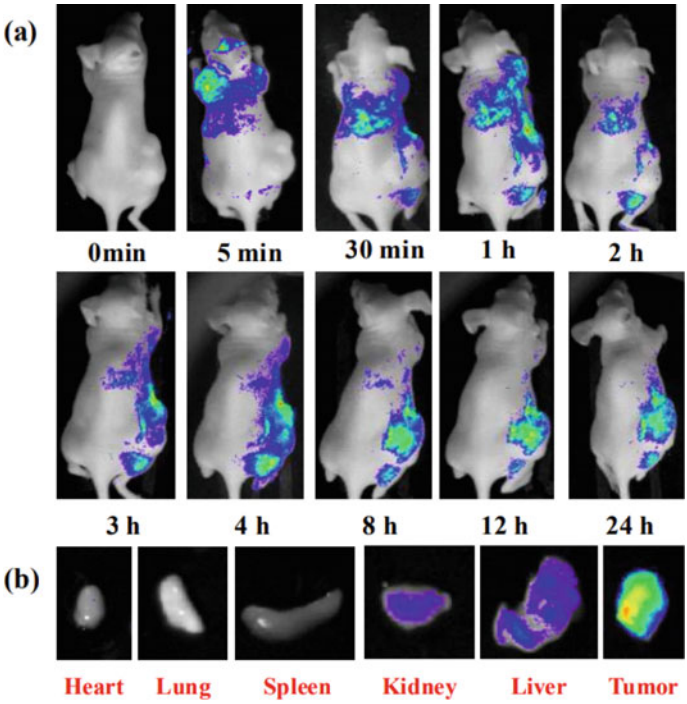

Fig. 3 **a** In vivo fluorescence imaging of nude mice was conducted following the intravenous administration of a solution containing colloidal quantum dot-tungsten sulfide (CQD-WS). **b** Illustrative fluorescence images of dissected organs from a mouse were obtained 24 h after the intravenous injection of a CQD-WS solution. reproduced with permission [17]. Copyright 2019, MDPI

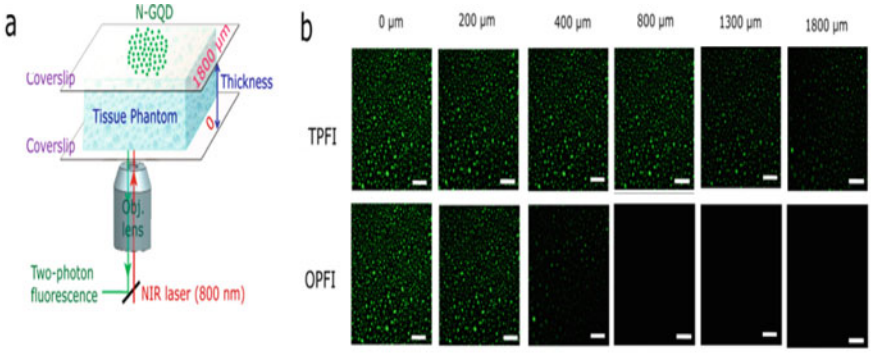

Fig. 4 **a** The diagram illustrates the arrangement utilized for conducting Two-photon fluorescence imaging (TPFI) of N-GQDs in a tissue phantom with varying thickness. **b** The depth of penetration of N-GQDs is presented for TPFI (on the right panel) and One-photon fluorescence imaging (OPFI) (on the left panel) within the tissue phantom. Reproduced with permission [18]. Copyright 2013, American Chemical Society

A-GQDs hold great promise as an emerging tool for a wide array of multifunctional biomedical applications [19].

Transition metal dichalcogenides (TMDs) are a family of layered compounds that Their three-atom thick unit cell is formed by a layer of transition metal atoms (Mo, W, Ta, etc.) sandwiched between two layers of chalcogen atoms (S, Se, Te) [20]. Among TMDs, molybdenum disulfide (MoS_2) has emerged as a promising candidate for these applications. MoS_2 can be synthesized as quantum dots (QDs) with a size range of 1–10 nm, which makes them suitable for biomedical applications [21]. MoS_2 QDNCs have several properties that make them attractive for drug delivery and imaging. First, they are water-soluble, which allows for easy dispersion in biological fluids and reduces toxicity concerns. They are also biocompatible and exhibit high stability, which makes them suitable for use in vivo. MoS_2 QDNCs also have a high surface area, which allows for high loading of drug molecules, and they can be functionalized with targeting moieties such as antibodies or peptides for targeted drug delivery. In addition, MoS_2 QDNCs have been shown to exhibit strong photoluminescence, which makes them useful for bioimaging applications. MoS_2 QDNCs also have other advantages over traditional semiconductor QDs. For example, they are less toxic than cadmium-based QDs, which are commonly used in bioimaging applications. MoS_2 QDNCs also exhibit a higher quantum yield and a broader excitation range than other TMDs such as tungsten disulfide (WS_2) QDNCs [22].

Roy et al. have designed a targeted bioimaging strategy to detect cancer cells that express the folate receptor (FR). This approach utilizes nanoprobes composed of free folic acid (FA) and MoS_2 quantum dots nanocomposites (QDNCs). The MoS_2 QDNCs were synthesized through a hydrothermal method, resulting in water-soluble particles measuring 4–5 nm with cysteine functionalization. Upon excitation at 370 nm, these MoS_2 QDs exhibit a blue emission with the highest intensity occurring at 444 nm. The MoS_2 QDNCs display a considerable level of sensitivity towards FA, rendering them a suitable choice for FA-targeted "turn-off" imaging probes. These probes can be effectively utilized in in vivo studies involving FR-overexpressed cancer cells that have undergone pretreatment with FA. Through the utilization of confocal microscopy, it was observed that B16F10 cancer cells, which were pretreated with FA, exhibited a higher population of dimmed fluorescence in comparison to both untreated cancer cells and HEK-293 normal cells. Additionally, flow cytometry analyses revealed a noteworthy disparity in the geometric mean of fluorescence between FA-pretreated B16F10 cancer cells and their untreated counterparts. These nanoprobes, based on MoS_2 QDNCs, have the potential to be utilized as nanoprobes for the pre-diagnosis of cancer via targeted bioimaging. The benefits of employing this method encompass the simple preparation of the nanoprobes, the exceptional selectivity and sensitivity of MoS_2 QDNCs towards FA, and the capability to achieve targeted bioimaging without the requirement of bioconjugating FA with nanoparticles [23].

Similar to MoS_2 QDNCs, WS_2 QDNCs have a size range of 1–10 nm, which makes them suitable for biomedical applications. They also exhibit unique electronic and optical properties due to their nanoscale size and quantum confinement effects. WS_2 QDNCs have several advantages over other types of QDs. For example,

they are less toxic than traditional cadmium-based QDs, which makes them more suitable for biomedical applications. They are also more stable under biological conditions, which makes them more suitable for in vivo imaging. In addition, WS_2 QDNCs exhibit strong photoluminescence, which makes them useful for bioimaging applications. They also have a large surface area, which allows for high loading of drug molecules, and can be functionalized with targeting moieties such as antibodies or peptides for targeted drug delivery [24].

Phosphorus is an essential element for maintaining good health in the human body, and the final degradation products of Black Phosphorus (BP) nanomaterials are harmless, making them suitable for use in biomedical applications [25]. There are three major allotropes of phosphorus, including white phosphorus (WP), red phosphorus (RP), and BP. WP, which has a tetrahedral structure, is chemically unstable, while RP possesses an amorphous structure and better chemical stability and BP has the most stable allotrope [26].

BP structures can be found in various forms, including 2D layered structures, 1D phosphorene nanoribbons, and zero-dimensional quantum dots. Unlike graphene, which has a zero band gap, and transition metal dichalcogenides (TMDs), which have a very large band gap, BP exhibits a tunable, direct, and thickness-dependent band gap. This property makes BP a promising candidate for a range of electronic and optoelectronic applications, including photovoltaics, transistors, and sensors. BP also has a sufficiently high carrier mobility, making it useful for high-speed electronics [27].

As a narrow band gap semiconductor, they are expected to show strong second near-infrared (NIR-II) fluorescence. Cholesterol-modified PEGylated BP nanoparticles (BP@lipid-PEG nanospheres) for in vitro on chicken slices and in vivo NIR-II imaging in normal nude mice were used by Xu et al. These nanospheres emit broad emissions from 900 to 1650 nm under an 808 nm laser, with a quantum yield of 8% of the standard dye IR-26. The NIR-II fluorescence image provides sharp contrast and can be used for blood vessel diameter measurements. Biocompatibility, biodegradability, and stability of nanospheres alongside gradually metabolizing by the liver in 48 h, making them promising for biomedical applications like imaging, drug delivery, and photothermal therapy (PTT) (Fig. 5) [28].

Post-imaging contrast agent's renal excretion poses a considerable challenge and may give rise to long-term toxicity. Specifically, contrast agents that are not readily eliminated from the body, such as certain agents employed in magnetic resonance imaging (MRI) and computed tomography (CT) scans, present a difficulty. Zhang et al. have synthesized black phosphorous quantum dots (BPQDs) possessing an ultra-small size (1.74 ± 0.23 nm) and modified with polyethylene glycol (PEG), thereby demonstrating suitable short-term stability (12 h). These BPQDs exhibit near-infrared (NIR) absorption increase and can produce robust photoacoustic (PA) signals when excited in the near-infrared. PEGylated BPQDs have the capacity to identify kidney dysfunctions, such as acute kidney injury (AKI) and chronic kidney disease (CKD), in living mice with a remarkable level of sensitivity and accuracy. This can be used in real-time observation for treatment response in kidney injury.

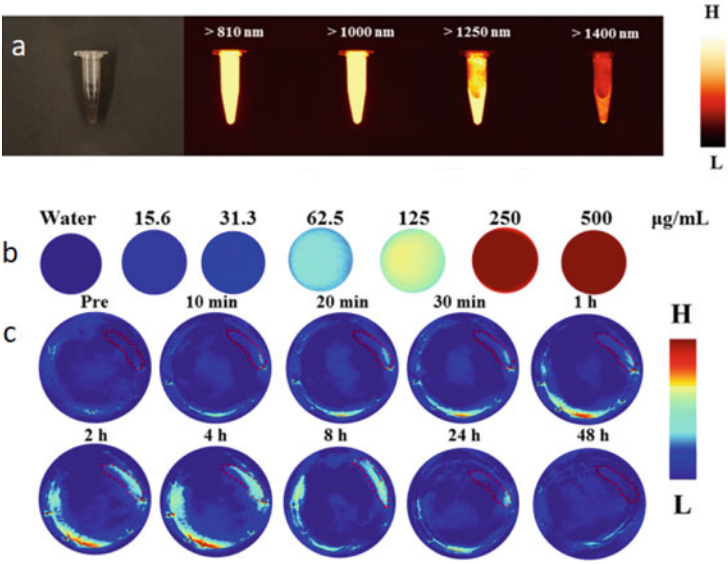

Fig. 5 **a** Optical images of a solution containing BP@lipid-PEG nanosphere, with a concentration of 1.3 mg ml^{-1}, were captured both in the absence and presence of irradiation by an 808 nm laser. **b** In vitro, photoacoustic (PA) images were obtained for aqueous solutions of BP@lipid-PEG nanosphere. c) Furthermore, in vivo PA imaging of the liver was conducted at various time intervals following intravenous administration of aqueous solutions of BP@lipid-PEG nanosphere to mice. Reproduced with permission [28]. Copyright 2019, American Chemical Society

Alongside of being biocompatibility and biodegradability, good renal-clearance of PEG-BPQDs improved depth of PA imaging [29].

4 Quantum Dots Nanocomposites Imaging Probe and PET Imaging

Fluorescence imaging is a useful technique for preclinical qualitative applications due to its high sensitivity, low cost, and short acquisition time. It has been used to provide intraoperative guidance with various fluorophore conjugated probes. However, fluorescence imaging suffers from limitations such as lack of quantitation, shallow depth of penetration, and tissue autofluorescence, which can limit its use in some applications. To overcome such limitation, another powerful imaging technique can be used alongside of fluorescence imaging. Positron emission tomography (PET) is widely used for diagnostic applications in clinical oncology. PET imaging utilizes radiolabeled compounds that emit positrons, which are detected by the PET scanner. This allows for highly sensitive and quantitative imaging, as well as the ability to image deep tissues within the body [30, 31]. When a positron (β^+) undergoes decay,

it produces high-energy positron particles that can travel through tissue at velocities greater than the speed of light in the same medium. This results in the emission of Cerenkov radiation, which is detectable by optical imaging techniques. However, Cerenkov radiation is highly absorbed in living organisms, meaning that only a small fraction of the emitted radiation can be detected. This limits the use of Cerenkov radiation for imaging applications in vivo. To overcome this limitation, researchers are developing new approaches to enhance the detection of Cerenkov radiation. One such approach is to use radioluminescent nanoparticles that can be excited by the Cerenkov radiation and emit light in the visible or near-infrared range. This allows for the detection of Cerenkov radiation with higher sensitivity and specificity [32].

Guo et al. used 64CuCl2 as precursor to synthesis [64Cu]CuInS/ZnS QDNCs. Positron-emitting radioisotopes attachment to QDNCs helps PET functionality and results in self-illuminating QDNCs, with no need for an external light source due to Cerenkov resonance energy transfer (CRET). [64Cu]CuInS/ZnS QDNCs showed great stability with less than 3% free 64Cu when exposed to serum containing Ethylenediaminetetraacetic acid (EDTA) for 24 h. To improve biocompatibility, radioactive QDNCs (RQDNCs) were PEGylated and showed higher tumor uptake in U87MG mouse xenograft model to a maximum of 10.8%ID/g. [64Cu]CuInS/ZnS QDNCs were successfully applied as an efficient PET/self-illuminating luminescence in vivo imaging agents [33] (Fig. 6).

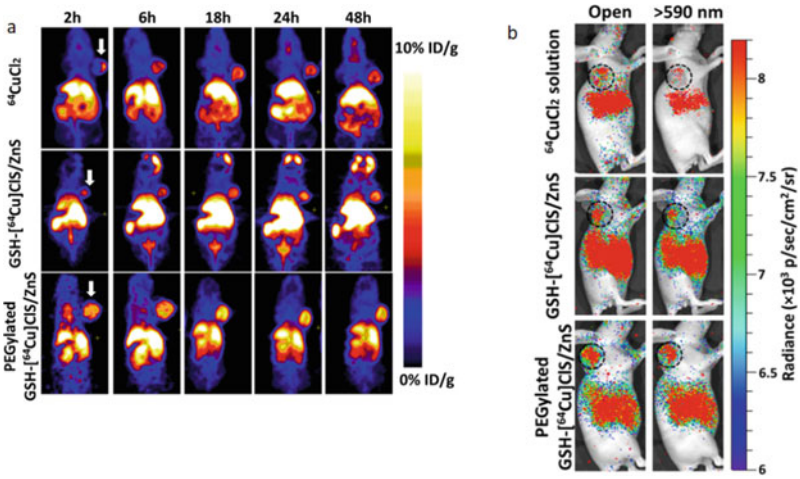

Fig. 6 A whole-body coronal PET images of U87MG tumor-bearing mice from 2 to 48 h were captured after intravenous administration of 100 μL (50 μg, 300 μCi) of 64CuCl2, Glutathione (GSH)-[64Cu]CIS/ZnS, and PEGylated GSH-[64Cu]CIS/ZnS RQDNCs (each group contained three mice's). The tumor location is indicated by an arrow. b) CRET images of U87MG tumor-bearing mice were obtained at 6 h after injection of 100 μL (300 μCi) of 64CuCl2, GSH-[64Cu] CIS/ZnS, and PEGylated GSH-[64Cu] CIS/ZnS RQDNCs. The tumor area was circled in these luminescence images (with 3 mice in each group). Notably, these images were captured without the use of excitation light, utilizing an open and red filter (>590 nm). Reproduced with permission [33]. Copyright 2015, American Chemical Society

Tu et al. synthesis dextran-coated silicon quantum dots (QDNCs) (d = 15.1 ± 7.6 nm, λ_{emi} = 440 nm) and labled with a macrocyclic ligand-64Cu2 + with 78% radiolabeling yield. Four mice were injected by radiolabeled QDNCs to investigate bio distribution in PET imaging and ex vivo gamma counting. Results showed that in a short period after injection QDNCs were accumulated in bladder and liver but after hours only liver showed strong signal. Ex vivo gamma counting approved in vivo imaging results [34].

5 Quantum Dots Nanocomposites Application in Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) is a noninvasive imaging technique that give high spatial resolution in clinical diagnoses. To obtain images with better and clearer resolution contrast agents can be helpful. Most of contrast agents that are used for MRI, are gadolinium (Gd3+) chelates such as gadolinium-diethylenetriaminepentaacetic acid (Gd–DTPA) and gadolinium −2,2′,2″,2‴-(1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrayl)tetraacetic acid (Gd-DOTA). These complex have high Gd^{+3} content and relatively low proton's longitudinal relaxivity (r1) which lead to high administration of them [35].

Conjugation of carbon quantum dots can be helpful to improve Magnetic resonance (MR) efficiency. Xu et al. developed carbon quantum dots nanocomposite -stabilized gadolinium hybrid nanoprobe (Gd–CQDNCs) that exhibit low toxicity and high biocompatibility on HepG2 cell line and mice model as in vitro and invivo toxicity assay respectively. Gd–CQDNCs exhibited a greater MR response compared to Gd–DTPA due to their elevated gadolinium concentration and hydrophilic properties. Additionally, there were no indications of the CQDs' fluorescence signal being compromised. The in vivo MR and fluorescence dual-modality imaging were tested on zebrafish embryo. Biodistribution of Gd-QDNCs was investigated in mice and showed no change in weight of mice's. The Gd–CQDNCs modification with arginine-glycine-aspartic acid (RGD) was done to enhance its binding affinity with U87 cancer cells, thereby facilitating targeted imaging. While the MR response exhibited a notable ability to penetrate depths and visualize spatial regions, fluorescence-based analysis provided a detailed distribution imaging of Gd–CQDNCs within tissues due to its superior resolution and sensitivity [36].

In another study Ding et al. anchord a single Gd atom on graphene quantum dots (SAGd-GQDNCs), which result in a dendrite-like structure and compared it to the commercial Gd-DTPA. MTT Test on HepG2 cells survive more than 90% and in vivo test on 4T1 tumor bearing mice showed that SAGd-GQDNCs had 25 times higher longitudinal relaxivity at low Gd^{+3} concentration as 2 μ mol/kg. The obtained result offer Gd-CQDNCs different complex form a potential carrier to be used as MRI contrast agent (Fig. 7) [37].

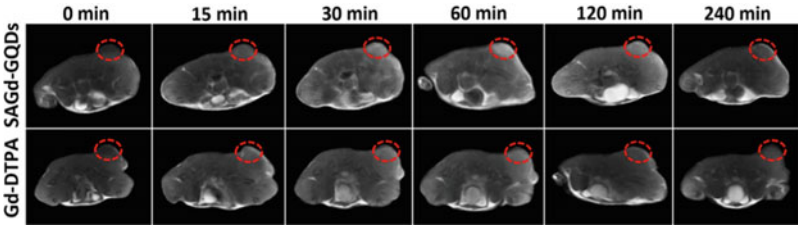

Fig. 7 In vivo magnetic resonance imaging (MRI) was performed on mice with tumors after an intravenous administration of two different samples, SAGd-GQDNCs and Gd-DTPA, at varying time intervals. The changes in the MRI signal of the tumor were indicated by a red circle. Reproduced with permission [37]. Copyright 2021, American Chemical Society

6 Conclusion and Future Perspectives

Quantum dots based nanocomposite (QDNCs) are amazing nanomaterials for diagnosis and bioimaging applications. The quantum dots specific chemical, physical and optical properties enable high functionalization capacity, excellent loading efficiency for imaging, sensing, diagnostic and theranostic application. Multimodal imaging or multiplexed imaging such as Photoacoustic/Fluorescence, MR/Fluorescence, PET/Fluorescence imaging and theranostic imaging application are the future of quantum dots based bioimaging. However, more animal and preclinical study should be done to understand the optimum dose of quantum dots and quantum dots nanocomposites without toxicity for clinical bioimaging application. However, quantum dots and quantum dots base nanocomposites offer a brilliant applicant for developing new bioimaging strategies and theranostic medicine as an imaging probe.

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Quantum Dots Nanocomposites as Drug Delivery Vehicle

Seyed Ahmad Dehdast and Omid Pourdakan

Abstract Quantum dots nanocomposites (QDNCs) has triggered enormous interest in, its applications in various fields of medicine and pharmaceutical science due to its unique properties. While QDNCs has demonstrated suitable potential as an imaging probe in medical imaging, its application as a nano-vehicle in drug delivery system for drugs and biomolecules in biomedicine, has only begun to be consideration. Advanced nano-carriers with slow, controllable release and excellent carrier efficiency are now considered the ideal vehicle for developing drug delivery systems. Remarkable properties of QDNCs include chemical stability, extremely large surface area, and low toxicity making it a promising material to design of novel drug delivery systems. Recent research has demonstrated significant success in the use of QDNCs for medical applications, including its utilization as a vehicle for controlled drug delivery, as well as in photothermal therapy, photodynamic therapy, and theranostic application. This chapter, presents a comprehensive overview of the current studies of drug delivery systems based on QDNCs vehicle. Moreover, various strategies for nano-vehicle design, functionalization methods for effective drug delivery, and reduction of quantum dots toxicity and the essential features for design of new Quantum dots nanocomposites vehicle in photothermal therapy, photodynamic therapy, and controlled drug delivery presented.

Keywords Quantum dots nanocomposites · Drug delivery system · Controlled release · Vehicle · Cancer · Photothermal therapy

List of Abbreviations

Ag	Silver
BBB	Blood-Brain Barrier
BPQD	Black Phosphorous quantum dots
BPQDs@Lipo	Black Phosphorous quantum dots liposome composite

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CdSe	Cadmium Selenide
CSCs	Cancer Stem Cells
CLSM	Confocal Laser Scanning Microscope
CMOS	Complementary Metal-Oxide-Semiconductor
CQD	Carbon Quantum Dots
DAPI	4′,6-Diamidino-2-phenylindole
DOX	Doxorubicin
EPR	Enhanced Permeability and Retention
FA	Folic Acid
FAR	Folate Receptors
FRET	Förster Resonance Energy Transfer
GQD	Graphene quantum dots
GOQD	Graphene oxide quantum dots
HP	HematoPorphyrin
IC50	Inhibitory concentration
In	Indium
InP	Indium Phosphate
MAP	Maximum Amplitude Projection
MDDS	Multi Drug Delivery Systems
MoS2	Molybdenum Disulfide
MUA	Mercaptoundecanoic
NIR	Near-Infrared
PA	Photoacoustic
PDT	Photodynamic Therapy
PEG	Polyethylene glycol
PLGA	Poly (lactic-co-glycolic acid)
PTT	Photothermal Therapy
QD	Quantum Dots
QDNC	Quantum dots nanocomposites
R&D	Research and Development
ROS	Reactive Oxygen Species
S	Sulfur
SOSG	Singlet Oxygen Sensor Green
TDD	Targeted Drug Delivery
WPU	Waterborne polyurethane
Zn	Zinc
ZnS	Zinc Sulfide
ZnSe	Zinc Selenide

1 Introduction

The major problems that threaten the health of people in the world today have created challenges and motivation for research and development (R&D) of various drugs and drug delivery systems using new methods. On the other hand, conventional medicine has some disadvantages such as poor bioavailability, weak targeting specificity, organ and systemic toxicity [1]. For this reason, R&D as to new drug delivery systems and therapies is growing and proceeding at an accelerated rate. The use of nanoscience and nanotechnology is one of the keys strategies of researchers to improve conventional treatment methods.

The use of nanoproducts requires detailed investigation due to the large chemical diversity and insufficient knowledge of these products. These studies should be designed in such a way that they provide detailed information about toxicity, biocompatibility, physiological effects, and the effects on other organs of the body in addition to the target organ. In case of appropriate response, the nanobiotechnology products can be used in clinical trials studies and possibly in treatment [2]. Quantum dots nanocomposites (QDNCs) are among the most popular semiconductor nanomaterials that have uniqueness physical and chemical, and optical properties such as High surface-to-volume ratio, high potential bio-chemical functionalization, photoluminescent properties, high surface energy, thermal, electrical, and optical features. These outstanding properties of quantum dots enable an extensive range of applications, such as optics, electronics, environmental science, and medicine. Quantum Dots and Carbon Quantum Dots have biomedical potential application, including diagnostic, bioimaging, antimicrobial, anticancer, drug delivery and, theranostic fields [3, 4]. The evaluation of benefits and challenges of utilizing quantum dots such as cytotoxicity, safety, inhibition concentration and drug release platforms in biomedical applications is important. Nanomaterials, including quantum dots, are taken up by various cellular methods, such as active and passive targeting, phagocytosis, and non-phagocytosis routes. Functionalization capacity of QDNCs and diversity in the entry routes of nanomaterials into the cell makes it possible to design different drug carriers with different strategies for drug delivery and therapy [5]. Recently, several studies have been conducted based on QDNCs drug delivery systems for various diseases treatment such as malaria, cancer, etc. This chapter offers an exciting overview of recent researches of this emerging area for researchers in the fields of medicine, biomedical engineering, pharmaceutical science and material science, as well as for nanomedicine groups. In this chapter, we discuss about Quantum dots nanocomposites based drug delivery systems that have been studied in vitro and preclinical studies.

2 Controlled Drug Delivery

Controlled drug release is a strategy used to regulate the rate and timing of drug delivery to a specific target site in the body. This approach is particularly useful in cases where sustained drug exposure is required, or when drugs have a narrow therapeutic window and must be delivered in precise doses.

There are several ways to achieve controlled drug release, including the use of drug delivery systems such as nanoparticles, graphene quantum dots, liposomes, and hydrogels. These systems can be designed for drugs release in response to specific stimuli, such as light, pH changes, temperature, and the presence of enzymes or other biomolecules [6, 7].

Near-infrared (NIR) light has acquired popularity in biomedical applications because of its non-invasive nature and its capability to penetrate tissues with minimal absorption and scattering. NIR light has a wavelength range of 700–2500 nm, which allows it to pass through biological tissues with minimal damage, making it an attractive option for non-invasive diagnostic and therapeutic applications.

In addition to its tissue-penetrating ability, NIR light can be adjusted using a range of parameters, including wavelength, duration, and intensity, enabling precise control over the amount of energy delivered to a specific target site. This makes it possible to tailor the light to the specific needs of the application, whether it's for imaging, therapy, or a combination of both [8, 9].

Geng et al. put black phosphorus quantum dot in the hydrophobic bilayer and Doxorubicin (DOX) in liposome aqueous core to controlled release of drug by NIR light irradiation. Biocompatibility on two normal and cancer cell line L929 (mouse fibroblast cells), QSG-7701 (normal human liver cells), A549 (human lung carcinoma cells) and MCF-7 showed good biosafety. The released rate can be controlled by laser power, means with increase in power release rate increase also. The in vitro therapeutic assessment showed that group which incubated with DOX-loaded black phosphorous quantum dots liposome composite (BPQDs@Lipo) and irradiate with 808 nm laser for 10 min achieved maximum cell death among other groups result in promising efficiency of this chemo-photothermal therapy (10).

Liu created a versatile nanocarrier, Mo_S2-PEG, by modifying highly fluorescent MoS_2 QDs with biocompatible Polyethylene glycol (PEG). This modification resulted in improved physiological stability and decreased toxicity. MoS_2-PEG-DOX, the resulting drug carrier, demonstrated desirable pH-responsive drug release properties. Results from a confocal laser scanning microscope (CLSM) study showed that DOX detached from MoS_2-PEG inside the cytoplasm and entered the nucleolus to inhibit cellular growth. The cellular uptake of MoS_2-PEG and drug release can be monitored simultaneously using two separate fluorescence channels, which reveal their localization and relative internalization kinetics (Fig. 1). These findings suggest that pH-responsive drug delivery systems based on MoS_2 QDs may be a promising option for cancer therapy [11].

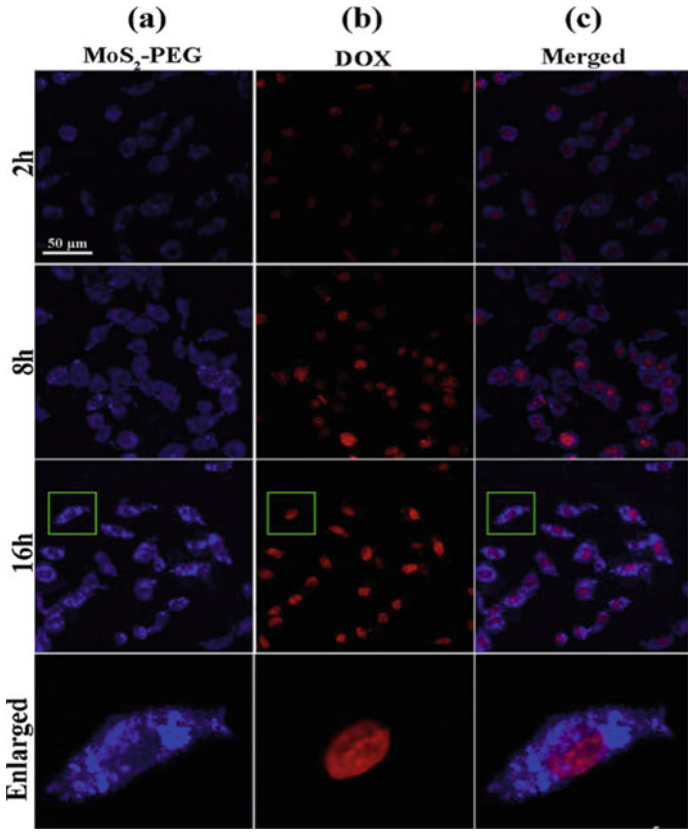

Fig. 1 CLSM images were acquired of U251 cells that were subjected to incubation with MoS_2-PEG-DOX for varying periods of time. The obtained images encompass three distinct categories: **a** MoS_2-PEG, **b** DOX, and **c** merged images of (**a**) and (**b**). Reproduced with permission [11]. Copyright 2020, Elsevier

To increase the effectiveness of chemotherapy in killing cancer cells, it is beneficial for large doses of anticancer drugs to enter the nuclei of cancer cells. Su et al. cancer-stem-cell-nucleus-penetrable red-emissive carbon quantum dots (CSCNP-R-CQDs) that are capable of entering the nuclei of both cancer cells and cancer stem cells due to nitrogen surface group. When doxorubicin was loaded onto the surfaces of the carbon quantum dots at a concentration of 30 μg/mL, the average viability of HeLa cells was reduced to 21%, while free doxorubicin only decreased cell viability to 50% (Fig. 2). The doxorubicin-loaded carbon quantum dots also showed promise in eliminating cancer stem cells. This study suggests that carbon quantum-dot-based drug carriers have potential for effectively eradicating cancer and represents a potential strategy for developing new anticancer drugs [12].

Pancreatic cancer has a poor prognosis and is resistant to traditional therapies, making it the most deadly of all cancers. Nigam et al. investigate the potential of

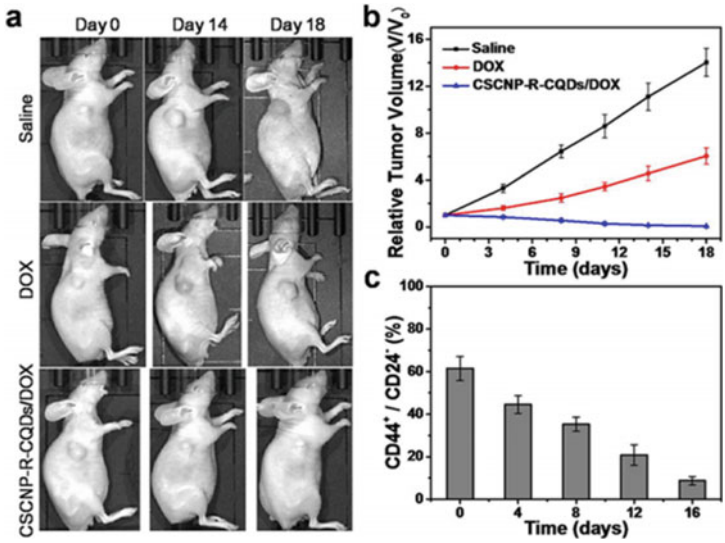

Fig. 2 **a** Exhibit a tumor-bearing mice images after treatment with saline solution, DOX, and CSCNP-R-CQDs/DOX on various days. **b** The relative volume of tumors in the mice was measured following the administration of diverse treatments. **c** Furthermore, the percentage of Cancer Stem Cells (CSCs), specifically CD44+/CD24− levels, present in the cells obtained from the tumors were also measured after 0, 4, 8, 12, and 16 days of treatment. All the provided data is presented as the mean value ± standard deviation, with a sample size of n = 5. Reproduced with permission [12]. Copyright 2020, American Chemistry Society

hyaluronic acid functionalized and green fluorescent graphene quantum dot (GQD)-labeled human serum albumin nanoparticles for pancreatic cancer-specific drug delivery and bioimaging. GQDs have become more popular than their metal semiconductor counterparts due to their tunable fluorescence properties and biocompatibility. The synthesized GQDs had excellent fluorescence and a good quantum yield of approximately 14%. Gemcitabine, the preferred drug for pancreatic cancer treatment, was encapsulated in albumin nanoparticles. The presence of hyaluronic acid as a targeting moiety on the human serum albumin nanoparticles improved the efficiency of gemcitabine on resistant pancreatic cancer cells. The nanoformulation significantly enhanced the bioavailability and sustained release property of the drug to pancreatic cancer cells in vitro [13].

Lung cancer is a common and deadly form of cancer worldwide, and conventional chemotherapy is the primary treatment option for small cell lung cancer. However, it lacks specificity and often results in severe side effects [14]. To address this issue, Ruzycka-Ayoush developed a new targeted drug delivery (TDD) system using Ag–In–Zn–S quantum dots (QDs) modified with various molecules, including folic acid (FA), to target doxorubicin (DOX) to folate receptors (FARs) on adenocarcinoma human alveolar basal epithelial cells (A549). The QD–FA–DOX nanoconjugates showed higher cytotoxicity than other synthesized QD samples. In contrast,

QD–MUA–FA–DOX nanoconjugates were the most cytotoxic against A549 cells, confirming FARs as molecular targets. The QD–MUA–FA–DOX nanoconjugates also significantly inhibited A549 cell migration in an in vitro scratch assay. Furthermore, all nanoconjugates induced significantly more DNA breaks than non-treated cells at the IC_{50} dose (Fig. 3). Overall, the QD–MUA–FA–DOX nanoconjugates showed the greatest cytotoxicity and genotoxicity and significantly inhibited the migratory potential of A549 cells [15].

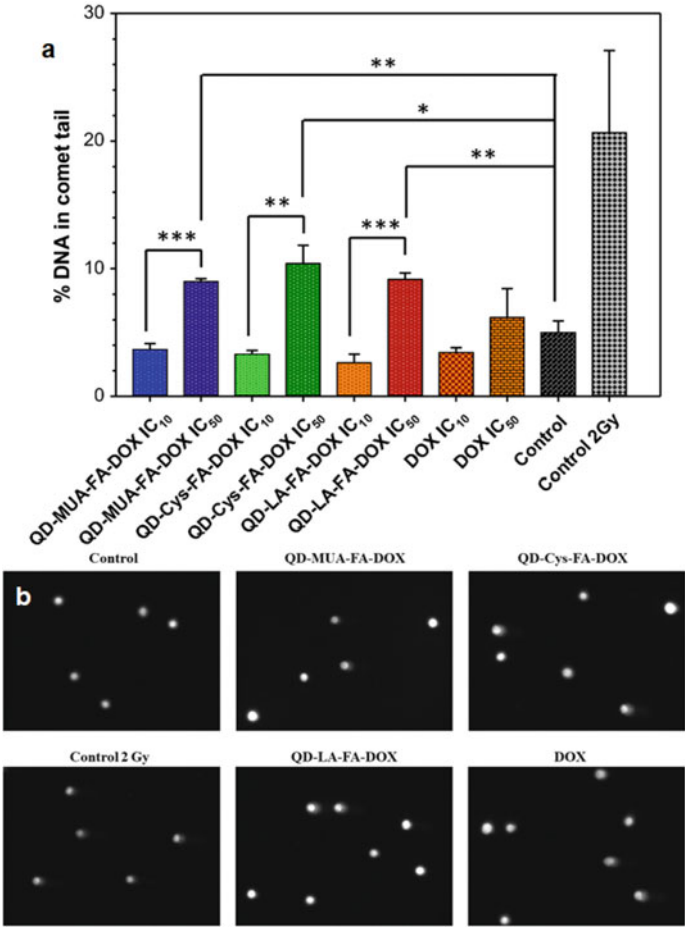

Fig. 3 **a** Represent the DNA damage percentage in A549 cells after treatment with QD–MUA–FA–DOX, QD–Cys–FA–DOX, QD–LA–FA–DOX, and DOX at IC10 and IC50. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, and $****P < 0.0001$, between the investigated concentrations (unpaired t-test). **b** Fluorescent images were obtained of the DNA damage of A549 cells subsequent to incubation with the compounds at IC50 after 24 h. Damage detected by the comet assay. Ag–In–Zn–S QD modified with: 11-mercaptoundecanoic acid (MUA), Cys L-cysteine, LA lipoic acid, FA folic acid, DOX doxorubicin. Reproduced with permission [15]. Copyright 2021, Springer Nature

3 Photothermal Therapy

Alzheimer's disease is a progressive neurodegenerative disorder that affects the brain, leading to memory loss, cognitive decline, and behavioral changes. It is the most common cause of dementia and typically affects people over the age of 65, although early-onset forms of the disease can occur in younger individuals. The exact cause of Alzheimer's disease is not yet fully understood, but it is thought to involve a combination of genetic, environmental, and lifestyle factors [16]. Multiple factors are in response for this disease but important to mention are beta-amyloid plaques, tau tangles and ROS production enhancement. Blood–brain barrier (BBB) hardly let drugs to pass, result in low efficacy of drug [17]. Qi et al. developed a MoS_2-QDs as carrier and functionalized with lipid containing curcumin to overcome poor water solubility and bioavailability of drug and reduce Aβ1-42 aggregation and eliminate ROS. QD-drug complex showed great potential when combined with NIR treatment and reduced Aβ1-42 mediated neurotoxicity. Photothermal experiment on mices showed that NIR radiation can alter BBB and increase its permeability. It was observed that temperature difference between mice's brain increased and reflect MoS_2 QDs/lipid photothermal property [18].

Bao et al. developed S, N-doped carbon dots (CDs) which exhibited remarkable effectiveness in photothermal therapy (PTT) when tested on mice. These CDs exhibited an impressive conversion efficiency of 59% in aqueous solutions when subjected to 655-nm laser irradiation. The unique biodistribution and targeting of near-infrared (NIR) CDs enhance their potential as theranostic agents. The CDs exhibited a low level of cytotoxicity and were eliminated from the body through the renal filtration system. Moreover, upon intravenous administration, the NIR CDs passively targeted and accumulated in tumor tissue, obviating the requirement for active entities such as antibodies. This resulted in robust NIR fluorescence and photoacoustic signals. The tumors were eradicated after intravenous administration of the CDs, followed by exposure to a 655 nm laser. The CDs' accumulation in tumor tissue without the need for intratumor injection, their high photothermal conversion, exceptional optical and photoacoustic imaging capabilities, and renal excretion render them well-suited for utilization in clinical biomedical practice (Fig. 4) [19].

Perini et al. found that graphene quantum dots (GQDs) with surface carboxyl groups can increase membrane permeability in a 2D and 3D model of glioblastoma multiform, a type of brain cancer. This enhances the effectiveness of two chemotherapeutic drugs, doxorubicin and temozolomide, even when administered at sub-therapeutic doses. The proposed mechanism of action is that the increased membrane permeability induced by GQDs improves the uptake of antitumor drugs inside glioblastoma cells. The study suggests that biocompatible GQDs can increase membrane permeability through photothermal conversion in a reliable tumor model, allowing the use of sub-therapeutic doses of antitumor drugs, enhancing their efficacy specifically in cancer regions while reducing side effects, potentially improving the quality of life for patients. Additionally, the increased membrane permeability due to GQDs-mediated PTT enhances the release of reactive oxygen species, resulting in

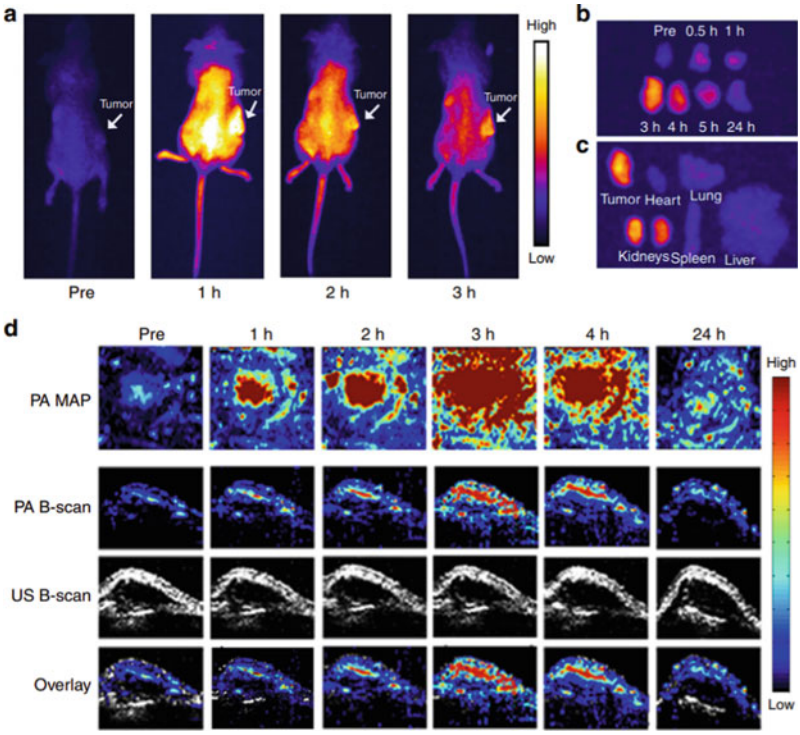

Fig. 4 Exhibit in vivo CDs that passively targeted. **a** Mouse bodies NIR fluorescence images were captured after the intravenous administration of CDs at different time intervals. **b** The near-infrared fluorescence of H22 tumors that were removed from mice at different time intervals after injection were analyzed. **c** The near-infrared fluorescence of major organs and H22 tumors that were dissected from mice three hours after injection is measured. The near-infrared fluorescence images of the mouse bodies, major organs, and tumors were captured using a 655 nm laser with an intensity of 10.6 mW cm^{-2}. The emitted light was filtered using a 700 nm long pass filter and recorded using a CMOS camera for near-infrared imaging. **d** 4T1 tumors Photoacoustic (PA) maximum amplitude projection (MAP) and B-scan PA images in mice were obtained after the intravenous injection of CDs at different time points. Reproduced with permission [19]. Copyright 2018, Nature Publication Group

improved efficacy of immunotherapy with strong migration of the immune system towards irradiated cancer spheroids [20].

Shao et al. developed biodegradable nanospheres by loading black phosphorus quantum dots (BPQDs) into Poly (lactic-co-glycolic acid) (PLGA). BPQD rapid degradation was prevented due to PLGA hydrophobicity, improving their photothermal performance in a physiological environment. The nanospheres showed no significant toxicity and good biocompatibility in hematological, blood biochemical, and histological analyses. The nanospheres exhibit prolonged blood circulation and efficacious tumor accumulation upon intravenous administration through the enhanced permeability and retention (EPR) effect. Remarkably, even in deep tissues,

the nanospheres effectively induce apoptosis in cancer cells when subjected to near-infrared (NIR) laser irradiation, leading to highly efficient tumor ablation. Furthermore, the BPQDs/PLGA nanospheres have exhibited efficacy in animal models with different immune systems. After fulfilling their therapeutic function, the nanospheres degrade and release BPQDs that transform into nontoxic phosphate and phosphonate compounds, which are subsequently cleared from the body. This gradual degradation of BPQDs circumvents any local phosphorus imbalance invivo, highlighting the clinical potential of BP-based photothermal therapy as a uniquely biodegradable and biocompatible agent [21].

4 Photodynamic Therapy

Dong et al. synthesize MoS_2 quantum dots to compare their ability to generate oxygen for photodynamic therapy with commercial photodynamic agent PpIX. Cytotoxicity on HeLa cells showed no obvious result on cell which indicate good biocompatibility of particles. In comparison to MoS_2 QDs, PpIX showed 22% decrease in absorption but QDs showed photostability after 1h irradiation. To evaluate the $^1O_2$ production within cells, a singlet oxygen sensor green (SOSG) reagent was used. QDs showed stronger fluorescence signal which was a sign of more oxygen production compare to PpIX which provide great promising application in photodynamic therapy [22].

Black phosphorus quantum dots (BPQDs) are of interest in cancer therapy due to their excellent biocompatibility and photothermal and photodynamic efficacies. However, their low environmental stability and poor dispersity have limited their practical applications. Lu et al. synthesized, biocompatible anionic water-borne polyurethane (WPU) nanoparticles from castor oil to encapsulate BPQDs, resulting in much-improved dispersion and environmental stability without affecting the photothermal efficiency of BPQDs. Interestingly, the encapsulation of BPQDs in WPU nanoparticles led to a significant enhancement in reactive oxygen species (ROS) generation, indicating an improved photodynamic efficacy compared to bare BPQDs. The pH regulation effect of WPU-BPQDs was investigated, and it was found that WPU-BPQDs boosted ROS production in acidic tumor environments compared to bare BPQDs. In vitro and in vivo experiments demonstrated that WPU-BPQDs effectively improved both photodynamic and photothermal therapy for cancer treatment. Overall, WPU-BPQDs have excellent dispersity, biocompatibility, and biodegradability and are a promising agent for photodynamic and photothermal cancer treatments [23].

Yan et al. created a biocompatible near-infrared (NIR) photosensitizer using a self-assembled CdSe quantum dot (QD)-aza-a@PEG conjugate for targeting tumors with concurrent imaging and photodynamic therapy. The aza-boron-dipyrromethene (aza-BODIPY) moiety in the conjugate emits strong NIR light at 750 nm upon excitation of the QD donor via efficient FRET, making it useful for bioimaging. Additionally, the conjugate can generate singlet oxygen for photodynamic therapy using laser light at 635 nm. The water-solubility of this conjugate and its ability to

target specific tumor cells is promising for various applications, including cellular labeling, deep-tissue imaging, assay labeling, diagnosis, and photodynamic therapy [24].

Ahirwar et al. demonstrated the efficacy of graphene quantum dots (GQDs) and graphene oxide quantum dots (GOQDs) in photodynamic therapy (PDT) for the eradication of cancer cells. GQDs and GOQDs were employed as photosensitizers for the PDT process, while 365 nm UV tube light was the irradiation source. The GQDs and GOQDs possessed a size range spanning from 1.5 to 5.5 nm and demonstrated photoluminescence behavior dependent on excitation, encompassing the UV to visible regions and yielding singlet oxygen. Furthermore, these QDs exhibited minimal toxicity towards skin cancer (B16F10) and breast cancer (MCF-7) cells, along with facile cellular uptake and distribution within the cells, thus positioning them as promising candidates for PDT application. Photodynamic therapy (PDT) was conducted on B16F10 and MCF-7 cells utilizing GQDs/GOQDs, resulting in a remarkable mortality rate of over 90% within a brief exposure period of 5 min. Conversely, cells that were not subjected to GQDs/GOQDs and were instead exposed to irradiation for the same duration exhibited a survival rate of more than 80%. GQD/GOQD PDT offers numerous advantages, such as its potent efficacy in killing cells through micro-watt power UV irradiation, reduced exposure time, and the ability to provide uniform and extensive coverage. Nonetheless, it is essential to acknowledge that the scope of this therapy may be confined to skin-related cancers due to the limited ability of UV irradiation to penetrate deeply into tissues [25].

The low solubility, poor photostability, and tendency to aggregate of conventional photosensitizers have posed obstacles to their application in photodynamic therapy (PDT) for cancer cells. This has led to the development of complex synthetic procedures, such as using nanomaterials to load photosensitizers, to effectively use photosensitizers in PDT. However, Murali et al. have developed a simple and efficient method for generating hematoporphyrin (HP) photosensitizer-encapsulated carbon quantum dots (CQDs) (HP-CQDs) through a one-step microwave reaction involving HP monomer as one of the precursor materials. The resultant HP-CQDs exhibited the same optical and chemical characteristics as HP, while also demonstrating exceptional solubility in water. Moreover, HP-CQDs displayed remarkable capability for producing reactive oxygen species under deep red light, which rendered them highly effective in the photodynamic therapy (PDT)-assisted eradication of human breast cancer cells (MCF-7), exhibiting significant phototoxicity and minimal dark toxicity in comparison to HP alone (Fig. 5). Overall it was demonstrates that that the integration of photosensitizer-implanted CQDs can be accomplished by carefully selecting appropriate precursors and synthesis conditions to yield CQDs, thereby offering a promising approach for PDT-assisted cancer treatment [26].

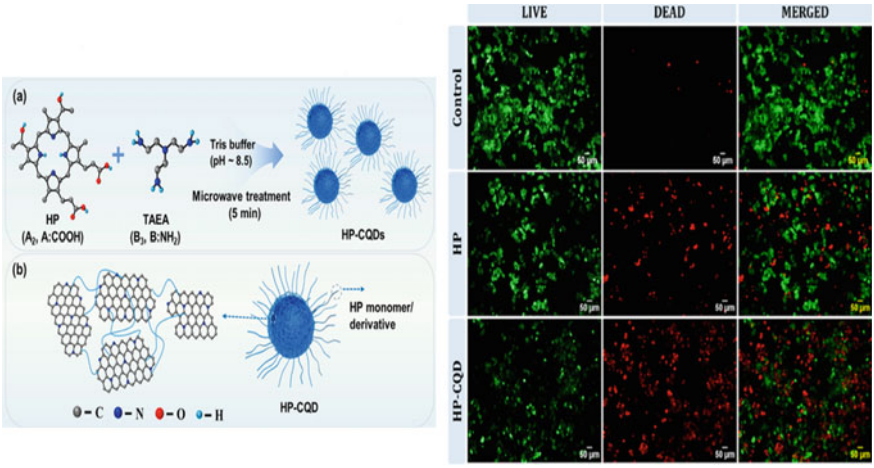

Fig. 5 Display schematic picture of **a** synthesis steps of HP-CQD and **b** structures that HP-CQD possibly can achieve through cross-linking in the left panel. A 635 nm laser at 50 mW cm^{-2} was used for 1 min to irradiate MCF-7 cells that were incubated with HP and HP-CQDs. After irradiation, cells were stained with green dye calcein-AM and red dye EthD-1 (red) to exhibit live and dead cells in confocal microscopy images, respectively, in the right panel. Reproduced with permission [26] Copyright 2022, American Chemical Society

5 Conclusion and Future Perspectives

Quantum dots nanocomposites are outstanding vehicle materials for drug delivery applications. The quantum dots specific chemophysical associated properties enable high functionalization capacity, excellent loading efficiency for imaging, diagnostic, controllable drug release, and drug targeting. Controlled drug delivery, multi drug delivery systems (MDDS) and theranostic application are the future of quantum dots and carbon dots based drug delivery.

However, more in vitro and in vivo and preclinical investigation must be performed to understand the biocompatibility, biosafety, toxicity and the long-term health effects of the associated with these nanocomposites. Finally, quantum dots based nanocomposites offer an excellent candidate for developing new nanodrugs and suitable vehicle for drug delivery systems in medicine.

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Quantum Dots Based Composites for Diagnostics

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Abstract In recent years, quantum dots (QDs) have emerged as a promising nanomaterial with the potential to revolutionize diagnostic applications due to their unique optical and electrical capabilities. In this setting, there has been a lot of interest in incorporating QDs into composite structures to create cutting-edge diagnostic tools and methods. This summary summarizes the present status of diagnostic composites based on Quantum Dots. It describes the fundamentals of QDs and how their fluorescence can be tuned and their properties vary depending on their size, making them promising candidates for sensitive and multiplexed detection. The synthesis, functionalization, and applications of QDs-based composites across multiple diagnostic platforms are all covered in this article. Their importance in improving the efficiency of in vitro and in vivo diagnostic techniques, such as biosensing, immunoassays, cellular imaging, and targeted drug delivery, is highlighted in particular. The difficulties and factors related to QDs-based composites' biocompatibility, toxicity, and clinical translation are also discussed in the abstract. Overall, this chapter highlights the revolutionary potential of Quantum Dots based composites in diagnostics, allowing for more precise, efficient, and individual monitoring of disease.

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1 Introduction

The remarkable and versatile optical features of quantum dots (QDs) make them an intriguing family of nanoscale semiconductor materials [1]. The rules of quantum mechanics control the behaviour of these minuscule crystals, which typically range in size from 1 to 10 nm [2]. Due to quantum confinement effects, their properties deviate greatly from those of bulk materials, allowing for amazing applications in domains as diverse as electronics, photonics, and biomedicine. The electrical and optical properties of quantum dots vary with their size, making this one of their defining characteristics. Similarly to atoms and molecules, quantum dots have discrete energy levels rather than a continuous electronic state, as is the case with bulk materials [3]. The optical characteristics of a QD alter depending on its size because the bandgap (the difference in energy between its most occupied and least occupied energy levels) is sensitive to this difference. As a result, quantum dots exhibit size-tunable emission, a property that allows them to absorb and emit light at varying wavelengths. The high photoluminescence efficiency of quantum dots is also crucial. QDs undergo photoluminescence, in which they emit light at a larger wavelength after being excited by photons [4]. The nanoscale confinement of charge carriers leads to decreased non-radiative decay routes and enhanced radiative recombination rates, resulting in efficient light emission. Quantum dots are a promising technology due to their high photoluminescence efficiency, which has potential uses in a wide range of fields. Quantum dots may also absorb numerous photons of varying energy thanks to their broad absorption spectra [5]. Multiphoton absorption is a remarkable phenomenon that has paved the way for breakthroughs in fields like quantum computing and high-tech imaging. Exciting quantum dots with lower-energy photons via multiphoton absorption enables for deep-tissue imaging in biological systems with reduced risk of tissue injury [6]. Due to their extreme stability and suitability for long-term imaging applications, quantum dots are also a wonderful choice. Repeated stimulation causes fluorophores to gradually lose their fluorescence, a process known as photobleaching. However, quantum dots are resistant to photobleaching, meaning their fluorescence will be stable during your experiment [7]. The electrical characteristics of quantum dots are also unique. Applications in transistors, solar cells, and LEDs are possible due to their ability to function as efficient charge carriers in electrical devices [8]. Quantum dots are a possible contender for future electrical and optoelectronic devices because their electronic properties may be tailored for specific purposes by controlling their size, shape, and composition [9]. The possible toxicity of quantum dots is one of the problems that must be solved. There are worries regarding the effects of using some quantum dots made with heavy metals, including cadmium-based ones, on ecosystems and living organisms [10]. Quantum dots based on indium or carbon, which are less poisonous and more biocompatible, are among the alternatives being developed by scientists.

More than two thousand years ago, a method was established for synthesising PbS Qdots from inexpensive natural components including PbO, $Ca(OH)_2$, and water [11]. Hair dyes made from these ingredients were popular among Romans

and Greeks. One of the oldest and most often used methods for altering glass's hue is by manipulating the size of Qdots inside silicate glasses. CdS and CdSe were added to silicate glasses to produce red-yellow hues at the turn of the twentieth century. Using x-ray diffraction (XRD), Rocksby was able to determine in 1932 that precipitates of CdS and CdSe were responsible for the colours [12]. Doped glasses made from semiconductor particles were previously employed as filters in optics. In 1981, Ekinov and Onushchenko reported that nanometer-sized CuCl in silicate glass caused a blue shift in the optical spectrum [13]. In 1982, Efros and Efros proposed that the colour of glass might be modified by adjusting the size or stoichiometry of CdSxSe1-x due to quantum size effects (the change of optical and optoelectronic properties with size) [14]. In 1991, Rosetti et al. discussed the topic of colour shifts in semiconductor colloidal solutions [15]. There were a number of advances in synthesis over this time span [16]. Many basic features [17] of Qdots have drawn commercialization efforts [18], leading to a surge in experimental and theoretical study on these nanoparticles over the past two decades [2].

2 Optical Properties of Quantum Dots

QDs' unusual optical features are the result of their nanocrystal grain boundary and electron hole pairs (ex-citons). Among the many pho-tophysical properties of QDs are their resistance to photobleach and decreased fluorescence intermittency, as well as their high yields of fluorescent quan-tum and broad absorption and size-tunable spectra [19]. Due to their unique photo-physical properties, QDs have numerous potential applications in the biomedical industry, including but not limited to: diagnostics, medication delivery, gene therapy, etc.

Light emission properties that can be tuned by changing QD size are shown in Fig. 2. As was previously stated, the diameter of QDs is between 2 and 10 nm. By tuning their sizes, QDs emit light over a broad range of wavelengths, from 400 to 4000 nm, allowing them to be detected and identified in the visible, ultraviolet (UV), and infrared regions of the electromagnetic spectrum. As the diameter of the QDs is reduced, the degree of confinement increases, leading to a larger bandgap and, ultimately, an exciton with a higher energy [20]. As a result of the high energy in the bandgap, the emission shifts to the low WL of blue spectra. Light WL is inversely proportional to energy. Quantum confinement causes electrons in nanomaterials to exist in discrete energy levels, as opposed to the continuous levels seen in bulk semiconductor material. The energy gap has of the QDs are changing with their dimension which is depicted in Fig. 1.

Absorption spectra are those produced when an atom absorbs enough photon energy (h) to jump from a lower to a higher energy level. When a molecule or atom emits a photon of energy, it is transitioning from a higher energy level to a lower one, and this process is known as the emission spec-tra. Due to the wide absorption band, numerous quantum dots can be stimulated by a single light source with WL less than emission WL. The aforementioned quality makes it easier to create fast and cheap

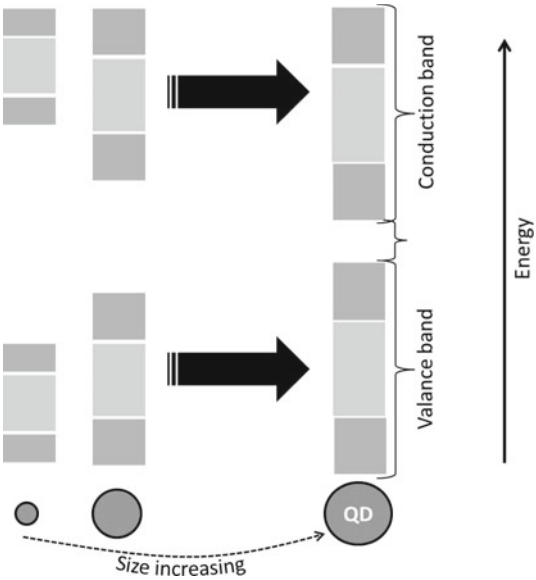

Fig. 1 The size-tunable optical characterization of QDs

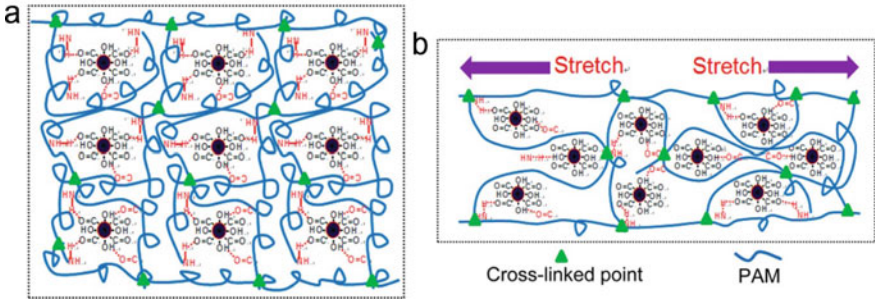

Fig. 2 Carbon dots reinforced superstretchable hydrogel microstructure in ambient condition and in stretched condition. Reproduced with the permission form Ref. [36]. © 2016 American chemical society

setups. By narrowing the QDs' emission spectra to 20 nm in the visible spectrum, we can identify individual signals without interference [21].

Photobleaching is the irreversible degradation of luminescent materials caused by light-responding processes or optical excitation, and it leads to a decrease in fluorescence intensity [29]. QDs, in general, exhibit a blinking characteristic under continuous stimulation of the molecule, which is an appealing phenom. This is because the QDs alternate between non-emitting and emitting states as a result of the trapping and detraining of photo-excited carriers. In contrast to fluoro-phores, the 'quantum

jumps' in intensity of fluctuations typically only last for brief intervals of time [22]. The crystalline shells of the quantum dots prevented them from blinking, though.

3 Properties and Advantages of Quantum Dots-Based Composites

The novel features of quantum dots (QDs) have attracted a lot of attention, and when QDs are embedded in composites, they open up exciting new avenues of research and application. These QD-based composites show a synergy of properties, combining the benefits of QDs and the host matrix, which leads to improved performance and multiple uses. Let's learn more about the benefits and features of composites made with Quantum Dots. QD-based composites have improved optical characteristics, which is one of their main advantages. The emission spectra of quantum dots can be tuned to produce a wide range of colors with great photoluminescence efficiency. These characteristics, when combined with a composite material, pave the way for the creation of cutting-edge optical devices including LEDs, displays, and sensors. Industries like electronics and photonics stand to benefit greatly from the brighter, more efficient gadgets made possible by the QDs' improved optical characteristics. Customized electrical conductivity can be achieved by incorporating Quantum Dots into conductive matrices and altering the composite's electrical properties. In terms of electronic activity, QDs can function either as charge carriers or charge traps. This property has applications in energy storage, flexible electronics, and conductive inks. Quantum confinement effects of quantum dots combined with conductivity of the host material can create composites with novel electronic characteristics. QD-based composites can also improve the mechanical properties of materials, such as their strength and flexibility. It is possible to increase the composite's mechanical strength, durability, and flexibility by tailoring the host matrix and QD size. This benefit can be put to use in a variety of settings, including structural materials, coatings, and even wearable technology, where improved mechanical durability and functionality are valued. Improved Sensing and Imaging: The photostability and brightness of quantum dots can be used to create very sensitive and precise detection systems when combined with sensor matrices or contrast agents. Bioimaging, medical diagnostics, and environmental sensing can all benefit from the use of QD-based composites [23–27]. QDs' adjustable emission wavelengths improve diagnostic platform throughput by enabling the simultaneous detection of several objects at once. Composites with specific thermal characteristics can be created by incorporating Quantum Dots into thermally conductive matrix. Applications like thermal management in electronics and energy-efficient materials benefit greatly from this property. The thermal conductivity of the composite can be precisely adjusted by altering the size and concentration of QDs. QD-based composites can be functionalized with a wide range of molecules including polymers, biomolecules, and nanoparticles, greatly increasing their potential applications. This adaptability paves the way for precise targeting, monitored

medication release, and a wide range of environmental interactions. Drug delivery and catalysis are just two of the many uses for composites based on QDs, which can be tailored to display specific chemical, biological, or catalytic functions. Quantum dots can be used as a greener alternative to more conventional materials in a number of different contexts [18, 28–32]. For instance, QDs can be used to lessen the environmental impact of paints and coatings by replacing pigments made with harmful heavy metals. In addition, QD-based solar cells and lighting systems show potential for eco-friendly and productive power production and use.

4 Types of QDs Nanocomposites

The optical or electronic properties of a quantum dot (QD) are determined by its inorganic semiconductor core, while the core is protected and functional groups for conjugation of biological probes are provided by the QD's organic coating layer. Cores can be formed from compounds like cadmium telluride (CdTe), cadmium selenide (CdSe), indium arsenate (InAs), cadmium sulfide (CdS), or lead sulfide (PbS), from which heavy metal ions can seep out and cause severe toxicity [20]. The introduction of QD into a biosystem raises serious concerns about the potential for core material-induced nanotoxicity. Despite recent advances in developing QDs that do not rely on heavy metals, their lackluster quantum yields (QY) and limited size-tunable emission range severely limit their usefulness in biomedicine [33].

4.1 QD-Polymer Nanocomposite

QDs are a potential new family of carbon nanomaterials due to their simple synthesis, unusual characteristics, and structural and compositional variety. QDs offer extensive surficial polar capabilities that, when combined with their other desirable properties, make them ideal candidates for further synthesis into QD/polymer nanocomposites, which can bring about significant performance increases and a host of new purposes.

Several polymers have been used to encapsulate QDs in an effort to lessen their cytotoxicity and make them more biocompatible. Diffusion inhibition of released Cd^{2+} ions from the QDs was shown to be the mechanism by which an amphiphilic polymer coating layer on CdSe QD greatly reduced QD cytotoxicity in an earlier study [34]. After encapsulating QDs in polyethylene glycol (PEG)-graftedpolyethylenimine (PEI), Nie and Duan discovered that the cytotoxicity decreased with increasing PEG grafts on each PEI molecule [38]. This finding indicates that polymer chains may wrap around QDs, creating a tight barrier that prevents the release of Cd^{2+} ions. Since PEI is a cytotoxic polymer, having PEG grafts present may reduce the likelihood of PEI molecules coming into contact with cells [35].

Fabricating hydrogels requires the use of both hydrophilic polymers and QDs with hydrophilic surface character. QDs have strong water dispersibility and minimal cytotoxicity because of their hydrophilic functionalities, which are typically hydroxyl, carboxyl, or epoxide functionalities. Hydrogels are in high demand as a soft material for biomedical applications, but their mechanical qualities are often insufficient for the purpose. Mechanical benefits (Fig. 2) were achieved through the use of CDs to alter the chemically crosslinked hydrogel network of polyacrylamide (PAM) [36].

Since CDs are known to be able to hold medicines with the help of intermolecular interactions and give rise to PL under UV/visible light, hydrogels incorporating CDs are promise for thermo-responsive drug administration with simultaneous bioimaging. Using the thermoresponsive polymer PNIPAM, CDs were revealed in a PAM network to create smart hydrogels that fluoresced blue at room temperature when exposed to UV light [37].

Polymer can improve QD biocompatibility, but it also has a number of limitations that reduce cell penetration capacity, water solubility, and imaging efficacy, such as increasing hydrodynamic size. Polymer coating layer may potentially significantly alter the physiochemical characteristics of the QD surface.

4.2 Silica-QD Nanocomposites

Since silica can inhibit flocculation of particles and preserve photoluminescence while also accepting addition of several functional groups like thiol, amine, phosphate, and carboxyl groups [38], it has been used to coat QDs. The Ströber method, a sol–gel procedure in which a silica shell grows with QDs as seeds in a water–ethanol mixture, has been used to create silica-encapsulated QD composites [39]. Nanocomposites made up of quantum dot (QD)/silica hybrid (Fig. 3) particles were studied for their resistance to high temperature and humidity in terms of their photoluminescence [40]. The maintenance of QDs' PL stability in the presence of heat and moisture is a challenge in their application as a color-converting material. Nanocomposites of QD/silica hybrid particles of varying morphologies were produced, and their PL stabilities in conditions of high temperature and humidity were studied. Additionally, they discovered that while photoactivation of QDs in the hybrid particles did improve the PL property at first, the PL decreased further due to subsequent photocorrosion.

In recent times, there has been a significant surge in research interest surrounding inorganic halide perovskite quantum dots (QDs) with the chemical formula $CsPbX_3$, where X represents the halide elements chlorine (Cl), bromine (Br), and iodine (I). This heightened attention can be attributed to the considerable potential these QDs hold for utilization in cost-effective light sources and displays. Nevertheless, the inadequate structural and chemical stabilities of these materials pose significant challenges that impede the reliable operation of devices. The present study achieved the successful synthesis of $CsPbBr_3$/silica quantum dot (QD) composites using a straightforward sol–gel reaction with tetramethoxysilane as the sole molecule precursor

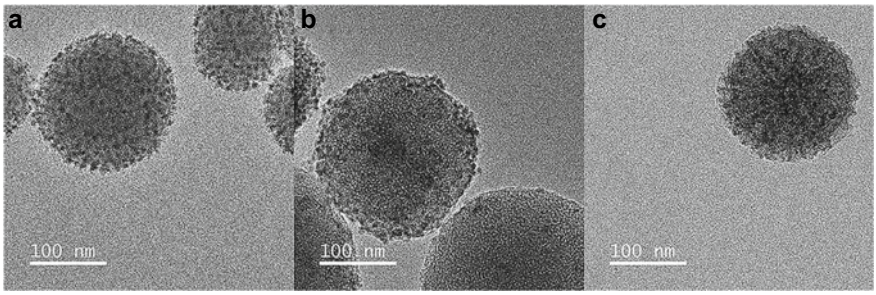

Fig. 3 TEM images of surface engineered QDs nanoparticles based on silica. Reproduced with the permission from Ref. [40] © 2020 American chemical society

[41]. The composites of $CsPbBr_3$/silica quantum dots, as prepared, exhibited significantly enhanced stability when exposed to heat, light, and environmental factors such as oxygen and moisture. In addition, the utilization of $CsPbBr_3$ QDs resulted in a comparatively reduced photoluminescence linewidth and an increased quantum yield when compared to the original $CsPbBr_3$ QDs. In their study, Jeon et al. employed silica particles as a template for the synthesis of silica–quantum dot–silica (SQS) core–shell–shell particles. They successfully regulated the refractive index of these hybrid particles by modifying their surface, aiming to reduce the refractive index disparity between the particles and the surrounding solvent and polymer matrix. The choice of silica particles as the template was motivated by their amenability to surface modification with a phenyl group, which is recognized for its substantial refractive index. The hybrid particles were employed for the production of a nanocomposite film, wherein poly(methyl methacrylate) was utilized as the matrix polymer. Subsequently, an analysis of their optical properties was conducted. It was observed that through the implementation of surface modification, enhancements were achieved not only in the transmittance but also in the mitigation of light scattering characteristics of the nanocomposite film [42]. Consequently, this resulted in an increase in the quantum efficiency (QE). In conclusion, nanocomposites incorporating hybrid particles and unmodified quantum dots (QDs) were utilized in the fabrication of a light-emitting diode (LED), and a comparative analysis was conducted to evaluate their respective efficiencies and stabilities.

The utilization of both silica and polymers in the modification of quantum dot (QD) nanocomposites has been employed in order to achieve the most desirable properties, as the surface chemistry of QDs significantly impacts their solubility, dispersity, optical properties, and probe conjugation. Polymer or silica matrices have been utilized to create a range of multifunctional nanocomposites incorporating quantum dots (QDs). This approach enables the encapsulation of multiple components within a single nanocomposite, thereby significantly broadening the potential applications of QDs in various fields such as bioimaging, biosensing, drug delivery, and cancer therapy.

5 Applications of Quantum Dots-Based Composites in Diagnostics

5.1 Drug Delivery

QDs have great potential as a medicine delivery and targeting technology. There has been a lot of excitement about the potential of QDs in the field of targeted drug delivery research. Their one-of-a-kind qualities have led to intensive research of them [43]. This evaluation took into account their significant function as medication delivery and targeting agents for anticancer medicines. This article provides a review of recent efforts to use QDs as a drug delivery and targeting system, with a special emphasis on their application in cancer therapy. Numerous research focus on the use of QDs in cancer therapy, despite the fact that multiple additional methods have also been lately presented for drug delivery. QDs have potential as useful instruments for microenvironment-specific medication delivery. Wei et al. assessed graphene QDs for this application [44]. One of the biggest issues in clinical practice is hypoxia-mediated chemoresistance in oral squamous cell cancer, thus they've produced Pt-loaded and PEG-modified graphene QDs (GPt) to combat this. In tumor biopsies, Pt biodistribution was observed, and more Pt was reported to accumulate in tumors after treatment with GPt than after treatment with free cisplatin. When considering the systemic effects, the Pt distribution in the kidneys and liver was also examined. In the GPt group, significantly less Pt was collected in the kidneys and livers compared to the free cisplatin group. The data demonstrated that oral squamous cell carcinoma cells were made more sensitive to therapy with GPt under both normoxic and hypoxic conditions, with the latter resulting in greater Pt accumulation in tumor cells. Furthermore, GPt was found to strongly limit tumor development while exhibiting far less systemic toxicity than free cisplatin. Microenvironment-specific cancer treatment with GPt has been proposed as an innovative approach. Researchers are looking into the synthesis of magnetic Fe_3O_4/SiO_2/graphene–CdTe QDs/chitosan nanocomposites (FGQCs) to see if they can improve the drug loading content over magnetic/fluorescent bifunctional nanocomposites [45]. The first observation of the in vitro interaction between the FGQCs and the heptoma cell line SMMC-7721 cells was conducted using TEM ultrathin section imaging. The graphene-quantum dots (QDs) demonstrate a significant luminescent response in aqueous surroundings when excited at a wavelength of 365 nm. The loading capacity and encapsulation efficiency of the fabricated graphene quantum dots conjugates (FGQCs) were determined to be 70% and 50%, respectively. Ina nother work the Fe_3O_4 nanoparticles were encased within the hyperbranched poly L-lysine citramid (HBPLC) [46]. Various techniques were employed to thoroughly characterize the prepared magnetic nanocarrier. The evaluative analysis focused on the diverse potential of the magnetic nanocarrier. The results of the in-vitro drug release studies indicate that the nanocomposite that was prepared demonstrates pH-responsive behavior. The study on antioxidants demonstrated the favorable antioxidant characteristics of the nanocarrier. Additionally, the nanocomposite exhibited a high level of photoluminescence, as evidenced by its quantum yield of 48.5%. The

results of cellular uptake investigations demonstrated that Fe_3O_4-HBPLC-Arg/QD exhibited a substantial level of cellular internalization in MCF-7 cells, indicating its potential utility in bioimaging applications. The nanocarrier that was prepared underwent in-vitro cytotoxicity, colloidal stability, and enzymatic degradability studies. The results indicated that the nanocarrier exhibited non-toxic properties, with a cell viability rate of 94%. Additionally, it demonstrated stability in colloidal form and a biodegradability rate of approximately 37%. Javanbakht and Namazi presented a study on the fabrication of a novel hydrogel nanocomposite film incorporating graphene quantum dots (QDs) and doxorubicin (DOX) within carboxymethyl cellulose. Based on the findings, it was concluded that the nanocomposite hydrogel films possess potential as a viable platform for the delivery of anticancer drugs. This system has the capability to achieve pH-dependent and prolonged release of DOX [47]. Sesamol exhibits anticancer properties and demonstrates a notable reduction in tumor size and lipid peroxidation levels within cancerous cells. Nevertheless, due to its water solubility, sesamol encounters substantial challenges in terms of cellular penetration and internalization. A study was conducted to synthesize CdS-modified chitosan with the aim of enhancing the bioavailability of sesamol. The system under investigation was effectively loaded with sesamol, resulting in a notable enhancement of the cytotoxic activity associated with sesamol [48].

The incorporation of inorganic nanoparticles and anticancer agents within polymeric nanoparticles, resulting in a core–shell structure, offers several advantages. Firstly, it enhances localized contrast, enabling improved visualization through various diagnostic techniques, thereby facilitating precise diagnosis. Additionally, this encapsulation strategy ensures heightened therapeutic efficacy, leading to increased effectiveness in cancer treatment [49]. In the present context, quantum dots (QDs) have undergone thorough assessment for their potential in theranostic applications, specifically in the field of cancer diagnosis and therapy, as demonstrated by numerous researchers. The present study introduces a multifunctional drug delivery system that consists of two inorganic nanocrystals and an anticancer drug, all of which are enclosed within a biodegradable poly (lactic-co-glycolic acid) (PLGA) shell. In this study, inorganic nanocrystals, specifically superparamagnetic iron oxide nanoparticles (SPION) for in vivo magnetic resonance imaging (MRI) and cadmium-free manganese-doped zinc sulfide (Mn: ZnS) quantum dots (QDs) for fluorescence in vitro imaging, were employed. The selection of busulfan as a lipophilic anticancer agent was made.

In a separate investigation, a nanocomposite comprising Fe_3O_4/CQD was synthesized, exhibiting combined magnetic and fluorescent characteristics [50]. In the initial step, the Fe_3O_4 nanoparticles were synthesized and subsequently subjected to functionalization using glutaric acid. The surface of the nanoparticles was then modified to facilitate the attachment of CQDs, as depicted in Fig. 4a. The utilization of magnetic/fluorescent nanocomposites (Fe_3O_4/CQDs) has facilitated the incorporation and application of DOX in the field of medicine. The primary novelty of the current study lies in the multifunctional capabilities exhibited by Fe_3O_4/CQDs magnetic-fluorescent nanocomposites, which surpass those observed in prior research. These

nanocomposites demonstrate significant potential for utilization in medical diagnostics and therapeutics. The utilization of the nanocomposite as a contrast agent in magnetic resonance imaging (MRI) for diagnostic purposes is facilitated by its possession of superparamagnetic characteristics. Furthermore, it possesses the ability to serve as a tool for the labeling of cancer cells and for optical imaging, owing to its inherent fluorescence characteristics. The utilization of Fe_3O_4/CQD nanocomposites for loading the DOX anti-cancer drug in therapeutic performance offers a controlled and targeted drug delivery system, as illustrated in Fig. 4b.

A conjugate, namely QD-MUC1-DOX, consisting of pH-responsive quantum dots (QDs), mucin1 (MUC1), and aptamer, has been developed and assessed for its potential in targeted drug delivery to ovarian cancer. The utilization of DNA aptamers, known for their specificity towards mutated mucin 1 that is overexpressed in ovarian cancer, involved their conjugation with QDs [51]. To ensure the stability of the system within the systemic circulation and facilitate drug release specifically within the acidic pH environment of cancer cells, the drug DOX was conjugated to QDs using a pH-sensitive hydrazone bond. This bond exhibits swift hydrolysis under acidic pH conditions. In a study conducted by Senel et al., N-doped graphene quantum dots were synthesized and subsequently coated with Eudragit RS 100 [52]. These QDs were further utilized as carriers for the loading of ephrin receptor-A2-siRNA. The system underwent testing to evaluate its performance in terms of DNA interactions, cell viability, antimicrobial efficacy, and antioxidant properties. The study unveiled that N-doped graphene quantum dots were rapidly taken up by the cells and exhibited

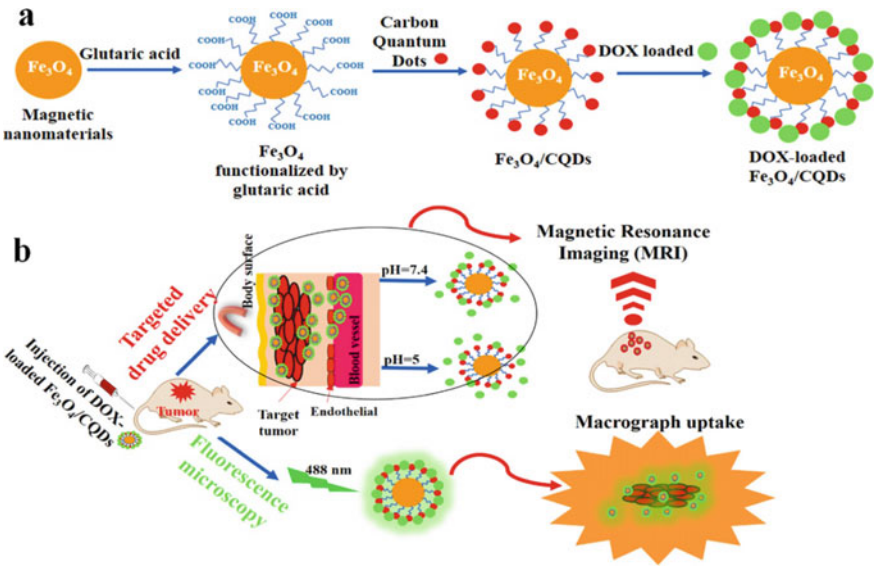

Fig. 4 **a** Preparation of DOX-Loaded Fe_3O_4/CQDs; **b** Applications of Nanocomposites in MRI, Targeted Drug Delivery, and Cellular Uptake. Reproduced with the permission from Ref. [50] © 2022 American chemical society

cell viability that varied with the dosage. Due to their inherent cell-impermeability, various strategies are required to enhance the translocation of quantum dots (QDs) across cellular membranes. Various techniques, including microinjection, electroporation, and lipofectamine-mediated transfection, have been examined as physical means to enhance the incorporation of quantum dots (QDs) into viable cells.

5.2 Immunoassays for Biomarker Detection

Due to their distinct optical features and adaptable functionalization options, quantum dots (QDs) have recently emerged as useful instruments in the field of biomarker detection using immunoassays. Immunoassays based on QDs, which utilize these tiny semiconductor particles, have the potential to radically alter the diagnostic and investigative processes. QDs are "sensitized" by having target-specific biomolecules, like antibodies or antigens, attached to their surfaces so that they can form a binding complex with the matching biomarker in a patient sample. Upon excitation, the QDs emit a unique fluorescence upon binding, providing a quantitative readout of biomarker presence and concentration. These immunoassays benefit greatly from QDs due to their remarkable fluorescence features, such as their size-tunable emission and high photostability. Multiplexing makes it possible to use a small number of samples to detect a large number of biomarkers in a single experiment by using QDs that produce light of different colors. Furthermore, QDs-based immunoassays are more sensitive than conventional approaches, allowing for the identification of low quantities of biomarkers. The fluorescence signal that is released can be measured, allowing for precise and precise analysis of biomarker concentrations. Additionally, immunoassays based on QDs can be included into microfluidic platforms and lab-on-a-chip devices, allowing for the downsizing, automation, and quick diagnostic procedures that these technologies enable. However, the importance of thorough safety assessments cannot be overstated when contemplating the translation of QDs-based immunoassays to clinical applications due to potential biocompatibility difficulties associated with various QD materials. Conclusion: Quantum dots-based immunoassays are a state-of-the-art tool for detecting biomarkers, heralding a sea change in clinical diagnostics, scientific inquiry, and the promise of game-changing developments in personalized medicine.

The use of bioconjugated QD nanocomposites to visualize cellular substructures in real time was reported by Kim et al. Here, we present a bioresponsive delivery system in which antibody-coated QDs are encapsulated in biodegradable polymeric nanospheres and undergo endolysosomal to cytosolic translocation (Fig. 5) via pH-dependent reversal of nanocomposite surface charge polarity [53]. The QD bioconjugates are released from the polymer nanospheres once they enter the cytoplasm and undergo hydrolysis. Without the need for cell fixation or membrane permeabilization, this method permits multiplexed tagging of subcellular components within living cells. This method permits high throughput cytoplasmic administration of QDs with negligible cellular toxicity, in contrast to traditional intracellular delivery

methods. This discovery also exemplifies a key rational approach to designing a nanosystem with multiple functions for use in biology.

CdTe quantum dots (QDs) and polymer nanocomposites are studied for x-ray scintillation and imaging. 510–680 nm aqueous CdTe QDs were integrated into polyvinyl alcohol or polymethyl methacrylate polymer matrices. Nanocomposite films had 5 lines/mm x-ray luminescent resolution. CdTe QD nanocomposites are promising for X-ray imaging due to their short decay time, nonafterglow, and excellent spectral match to charge coupled devices [54]. Lim et al. developed multispectrally encoded nanoprobes, perfluorocarbon (PFC)/quantum dots (QDs) nanocomposite emulsions, for multispectral MR and multicolor optical imaging. Our technique used four PFC materials' multispectral MR capabilities and three colored CdSe/ZnS QDs' multicolor emission properties [55]. PFC/QDs nanocomposite emulsions were made by exchanging hydrophobic ligands coated onto CdSe/ZnS QDs with 1H,1H,2H,2H-perfluorooctanethiol to make the QDs dispersible in PFC liquids. Phospholipids emulsified QD-containing PFC liquids into aqueous solutions for biocompatibility. Selective excitation of PFC/QDs nanocomposite emulsions with magnetic resonance

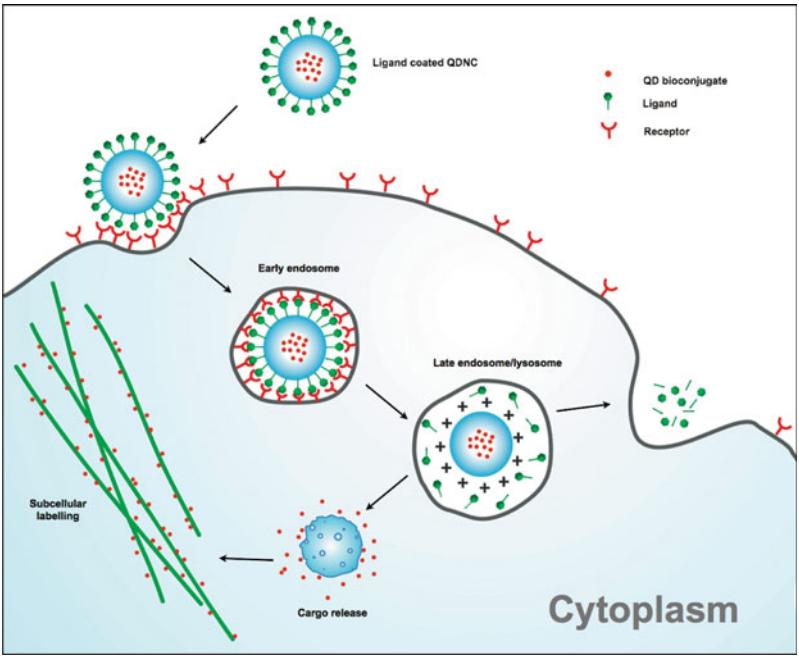

Fig. 5 QDNC cytosolic delivery and subcellular targeting. Schematic illustrating QDNC escape from the endolysosomal compartment and cytosolic release after cellular uptake. This method labels subcellular organelles and other molecular targets with antibody-conjugated QDs. *Note* QDNC charge distribution may be uneven inside acidic vesicles due to partial peptide bond breaking on the surface. Reproduced with permission from Ref. [53] © 2008 American Chemical Society

frequencies of each PFC yielded separate 19F-based MR pictures, while appropriate optical filters could pick a specific fluorescence image. PFC/QDs nanocomposite emulsions were taken up by macrophages (90.55%) and dendritic cells (85.34%), but not by T cells (33%). In vivo, nanocomposite emulsions differentiated immunotherapeutic cells (macrophages, dendritic cells, and T cells).

5.3 In-Vivo Diagnostics

Quantum dots-nanocomposites combine the unique features of quantum dots (QDs) with various nanomaterials to provide non-invasive, real-time biological process monitoring in live organisms. These hybrid structures combine QDs' size-tunable emission and long-lasting fluorescence with nanocomponents' multifunctionality. QDs-nanocomposites with biocompatible coatings and targeting ligands can be targeted to specific cells or tissues. These nanocomposites offer unprecedented imaging, disease detection, and treatment monitoring in vivo. QD fluorescence allows detailed imaging of cellular architecture and dynamics. QDs' tunable emission spectra allow multiplexing, which improves diagnostic accuracy by detecting many biomarkers simultaneously. QDs-nanocomposites' extended fluorescence and reduced photobleaching allow longitudinal research and real-time surveillance of disease development and therapy responses. Targeting ligands on QDs-nanocomposites ensure targeted contact with certain cells or molecules, reducing off-target effects. QDs-nanocomposites can encapsulate therapeutic molecules, function as diagnostic probes, and deliver drugs for image-guided therapy in theranostics. QDs-nanocomposites must be tested for toxicity, immunogenicity, and long-term consequences before being used in clinical settings. As the field advances, addressing biocompatibility concerns becomes crucial to unlocking the full potential of QDs-nanocomposites as a transformative tool for in vivo diagnostics, enabling a deeper understanding of biological processes, early disease detection, and personalized therapeutic interventions.

Another study generated biocompatible, photostable, and eco-friendly AgInZnS–graphene oxide (AIZS–GO) nanocomposites with tunable emissions by transferring hydrophobic AIZS QDs on water-soluble GO using a mini-emulsion approach [56]. The generated red emitted AIZS-GO nanocomposites showed promising uses in bioimaging and related domains, such as phototherapy and imaging, as demonstrated by in vivo imaging investigations of mice. The biomedical field could greatly benefit from magnetic-fluorescent nanocomposites, which could be used as a new dual-modality probe tool to improve the accuracy of medical detection. However, getting high-quality magnetic-fluorescent nanocomposites is difficult due to intricate and wasteful postprocesses. Therefore, it is still difficult to find a simple, efficient, and appropriate way for fabricating magnetic-fluorescent functional nanocomposites. The new synthesis methods are quickly becoming necessities, and they will likely allow us to solve the aforementioned issues [57]. Instead of using complex post-processing techniques, we describe a unique self-assembly synthesis method

for building magnetic-fluorescent bimodal imaging nanocomposites. Rapid cross-linking between Fe_3O_4 and QDs was achieved by cerium(III) ion induced coordination bonds, with cerium(III) ions acting as the connecting node and carboxylate groups as the bridging ligands. Mice with tumors were used to verify the potential utility of dual-modality imaging (Fig. 6). This clever method worked wonderfully for constructing the magnetic-fluorescent Fe_3O_4-QDs nanocomposite. Our cerium(III) ion driven self-assembly approach may have broad relevance for nanoparticles and organic molecules with carboxyl groups; however, further investigation is required.

Carbon nanohorns/chitosan/quantum dots (CNHs/CS/QDs) nanocomposite has been synthesized in a unique way. Amino-CNHs were encapsulated using CS to create amino-around surfaces, and they coupled with carboxyl-QDs in an effective manner [58]. The produced nanocomposite was then used for in vivo imaging of C. elegans and for marking HeLa cells, both of which relied on its robust red fluorescence. The nanocomposite's advantageous space property also makes it a promising candidate as a medicine delivery vehicle. Emerging as a useful tool for directing minimally invasive surgical operations is dual-modality imaging, which makes use of complementing modalities like all-optical ultrasound and photoacoustic imaging. Because of their wavelength-selective optical absorption, quantum dots are an exciting material for application in these dual-modality imaging devices. This paper presents the first quantum dot nanocomposite designed for simultaneous co-registration of laser-generated ultrasound and photoacoustic imaging [59]. The created nanocomposites are applied to the tips of tiny optical fibers; they are made up of $CuInS_2$ quantum dots and medical-grade polydimethylsiloxane (CIS-PDMS). High optical absorption (>90%) at 532 nm for ultrasonic generation and low optical

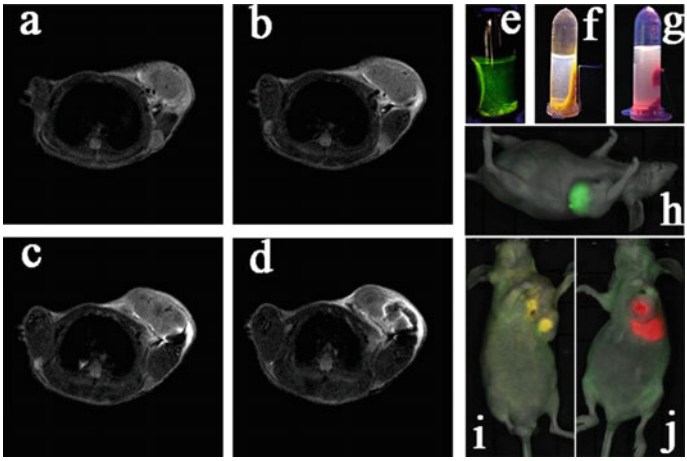

Fig. 6 In vitro/in vivo imaging. **a–d** MRI images. **e–g** Multicolor Fe_3O_4–QDs magnetic–fluorescent nanocomposites under UV illumination. **h–j** In-vivo multicolor fluorescence imaging. Reproduced with the permission from Ref. [57] © 2020 American chemical society

absorption (5%) at near-infrared wavelengths higher than 700 nm are just two examples of the films' wavelength-selective optical characteristics. Using the film's wavelength selectivity, an ink-filled tube phantom may be imaged using both all-optical ultrasound and photoacoustic techniques, which are then registered to one another. This study indicates the feasibility of using quantum dots as selective absorbers at specific wavelengths for producing all-optical ultrasound.

In order to act as both an imaging agent in the visible-light region and a photothermal cancer-therapy agent in the NIR region, a unique multifunctional, low-toxicity QD-rGO nanocomposite has been produced [60]. By using a surfactant layer to reduce QD toxicity and a carefully regulated spacer to minimize fluorescence quenching, this nanocomposite is able to solve several of the aforementioned challenges. Intriguingly, as the QD-rGO absorbs NIR irradiation, causing photothermal therapy and cell killing, the generated heat from the QD-rGO causes a temperature increase and a marked decrease in the QD brightness, providing a means for in situ heat/temperature sensing and an indicator of the progress of the photothermal therapy. In the meantime, coupling of the QD-rGO with targeting/internalization ligands has enabled active targeting and improved cell internalization.

6 Challenges and Limitations

While Quantum Dots (QDs) based composites show great promise for use in diagnostics, they still face a number of obstacles before they can be widely implemented in practice.

Issues of biocompatibility and toxicity have been raised in relation to the use of QDs in diagnostic settings. Heavy metals included in some forms of QDs may be harmful to living organisms. Avoiding negative effects on cells, tissues, and organs requires that QDs and the composite materials they are typically used with be biocompatible. Biocompatibility and possible buildup in live beings need extensive research to determine safety.

The stability, distribution, and clearance of QDs and their nanocomposites can be affected by the immune system's reaction to them. Long-term exposure to QDs in the environment may cause immunogenic reactions due to unfavorable interactions with the immune system. Preventing unwanted immune responses requires strategies to reduce immunogenicity and improve QDs' removal from the body.

Although QDs-based composites have shown promise in experimental experiments, there are still many obstacles to overcome before they may be used in clinical practice. Critical measures that must be performed to ensure safe and successful clinical deployment include regulatory approval, standardization of protocols, and validation of diagnostic performance.

For successful diagnostic and therapeutic applications, it is essential to achieve high levels of targeted specificity. It is a difficult task that involves accurate surface functionalization and careful design to ensure that QDs-nanocomposites reach the appropriate target cells or tissues while avoiding off-target effects.

Concerns have been raised about the long-term stability of QDs in biological settings. The accuracy of diagnostic tests may degrade over time due to aging-induced changes in optical characteristics, aggregation, or deterioration. Sustaining and reliable diagnostic results necessitate the implementation of strategies to improve the long-term durability of QDs-based composites.

Bright fluorescence is a hallmark of QDs, however the quantum yield and brightness of different QD formulations can vary. For effective and trustworthy diagnostics, it is essential to achieve uniform and optimum fluorescence emission across all batches of QDs.

Ethical and Regulatory Considerations The clinical application of nanomaterials such as QDs presents ethical and regulatory concerns. Potential effects on the environment, long-term safety, and patient consent are all important considerations.

Complexity and expense arise when manufacturing on a big scale, as in the synthesis and functionalization of QDs. One obstacle to wider implementation is the lack of established, low-cost methods for manufacturing QDs-based composites and incorporating them into diagnostic procedures.

The accuracy and specificity of QDs-based diagnostics can be impacted by interference and background signals, both of which can arise from complicated biological samples. Methods to reduce background noise and maximize signal-to-noise ratios are essential for producing accurate results.

Although QDs-based composites present an opportunity for quantitative analysis, it may be difficult to achieve precise and accurate quantification of biomarkers due to factors including differences in QD labeling efficiency and binding kinetics.

7 Conclusion

Quantum dots (QDs) integrated into composite structures have opened up novel diagnostic avenues and have the potential to radically alter the course of medical research and patient care. QDs' adaptability to functionalization and hybridization, along with their distinctive optical and electrical properties, have paved the way for a wide variety of ground-breaking diagnostic uses. The combination of nanotechnology and diagnostics has the potential to dramatically improve the efficiency, accuracy, and speed with which diseases can be identified, monitored, and treated.

QDs-based composites provide a flexible diagnostic toolkit for tackling complex problems in a wide range of fields. QDs have enabled the development of ultrasensitive biosensors and immunoassays, allowing for the quick and precise identification of biomarkers at trace levels in in vitro diagnostics. Multiplexed assays have been made possible by QDs due to their tunable fluorescence spectra and resilience to photobleaching, allowing for the simultaneous detection of many analytes in a single sample. The implications for improved diagnostic efficiency and thorough disease profiling are enormous.

Now, QDs-based composites are being used for in vivo diagnostics, allowing for the real-time viewing of cellular and molecular activities inside living creatures,

expanding the field of study far beyond the limitations of the traditional laboratory. Incorporating QDs into nanocomposites allows for precise functionalization and targeting, allowing for delivery to targeted organs and tissues. This paves the way for tailored treatment and monitoring, as well as non-invasive imaging of disease progression. Because of their versatility, QDs-nanocomposites are gaining increasing attention in the developing field of theranostics, where diagnosis and treatment are combined.

While QDs-based composites hold revolutionary diagnostic potential, we must first overcome obstacles related to biocompatibility, toxicity, and clinical translation. To ensure their feasibility and efficacy in clinical applications, these nanocomposites require rigorous studies of their safety profile. Successful clinical adoption of QDs-based composites will depend on addressing these problems through the integration of interdisciplinary research spanning materials science, biology, and medicine.

In conclusion, Quantum Dots based composites have sparked a paradigm change in the diagnostics landscape, providing a wealth of resources that reimagine our potential in the areas of early diagnosis, continuous monitoring, and individualized care. Nanotechnology combined with diagnostics has the potential to revolutionize healthcare by providing more precise, rapid, and tailored treatment plans for each patient. As knowledge of QDs-based composites increases, so does the scope of potential diagnostic innovations, highlighting the enormous promise that these cutting-edge technologies represent for the future of healthcare.

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Suitability of Hybrid Quantum Dots as Payload for Plants

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Abstract The use of hybrid quantum dots represents a unique opportunity to transform crop management and environmental monitoring through the integration of nanotechnology and agriculture. The combination of quantum dot characteristics with biological ingredients in these nanoparticles makes it possible to track plant health in real time, optimise nutrient uptake, and evaluate the surrounding environment. Hybrid quantum dots provide data-driven decision making, resource efficiency, and ecosystem preservation by delivering continual insights into plant conditions. However, issues like safety and cost need to be resolved before widespread use is possible. Nonetheless, hybrid quantum dots are a viable tool in the goal of smarter, more sustainable agriculture due to their potential benefits, which include enhanced crop yields and ecological sustainability.

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1 Introduction

It is expected that nanomaterials will play a significant role in helping the agricultural sector deal with these problems in the future. The agricultural sector has benefited greatly from the proliferation of nanomaterials over the past few decades, with their use facilitating the monitoring of crop health, the promotion of crop development, the improvement of fertiliser and pesticide efficiency, the management of diseases, pests, and environmental challenges, and so on. Because their carbon backbone is non-toxic, carbon-based nanomaterials have substantially less environmental toxicity and greater biocompatibility than metal-based nanomaterials. Therefore, nanoparticles made of carbon have found extensive use in farming. Carbon dots (CDs) are a novel type of carbon nanomaterial with intrinsic photoluminescence (PL) capabilities and a size of less than 10 nm. Due to their superior and universal properties such as high photostability, small size, biocompatibility, water solubility, highly tunable PL properties, easy functionalization with biomolecules, and chemical inertness, CDs have garnered a lot of attention from researchers in recent years. Carbon quantum dots (CQDs) have been the topic of extensive optical research studies for applications including biomonitoring, sensing, photocatalysis, medicine and gene delivery, solar energy conversion, and light-emitting diodes (LEDs), despite the fact that CDs were only discovered in 2004 [1]. As a result of the toxicity and environmental concerns associated with regular semiconductor quantum dots, CDs have been proposed as a replacement [2].

A synthetic structure with dimensions of 100 nm or less, engineered nanoparticles (ENPs) has unique physicochemical features like conductivity and optical sensitivity. (i) Semiconductor quantum dots (QDs), one of the significant ENPs, typically consist of elements from either group II–VI or III–V. Due to the quantum size effect (iii), QDs have distinct optical (ii) and electronic features, such as narrow and symmetrical emission, high photostability, and a strong Stokes shift, that are not seen in bulk materials. In the biological sciences (iv), QDs can be utilised as a replacement for the more common fluorescent dyes. (v) Biomolecule-templated synthesis, live organism synthesis, polymer-templated synthesis, bottom-up colloidal synthesis, and top-down synthesis approaches are some of the sophisticated QD synthesis methods for different pathways. Sixth, when synthesising highly dispersed QDs for bioapplications, bottom-up colloidal synthesis is the method of choice. Surface modification, often through ligand exchange procedures, surface silanization, and coating with cross-linked shells [3], allows QDs to acquire additional optimised features such as solubility, electrophoretic mobility, and targeted activities. Quantum dots (QDs) are often affixed to functional biomolecules (proteins, enzymes, or nucleic acids) by adsorption or covalent coupling for bioapplications including bioimaging or drug delivery systems.

The domestication of plants for subsistence purposes is among the most important human activities ever. The number of hungry individuals has dropped by half in the last 20 years because to improvements in agricultural production techniques [4]. However, there are still about a billion people who aren't getting enough to

eat. Agriculture requires an enormous quantity of resources to sustain the ever-growing population and accompanying demand from industry. An estimated 187 million metric tonnes of fertiliser, over 4 million tonnes of insecticides, and 2.7 trillion cubic metres of water (approximately 70% of all worldwide freshwater use) are used each year to produce the world's 3 billion metric tonnes of crops [5]. The widespread and inefficient use of pesticides and herbicides has led to significant resistance to these compounds, which poses a serious danger to biodiversity and the ecosystem. There will be additional pressure on food production and distribution because of climate change impacts like harsh weather and pest and disease epidemics. To meet the task of feeding a growing population, the agriculture sector must immediately adopt new eco-friendly technologies.

As a cutting-edge invention in nanotechnology, hybrid quantum dots are receiving attention for their potential to transform in different industry sectors [6–13]. The unique properties of quantum dots enable them to be used in promising applications [14–16]. We explore the exciting world of hybrid quantum dots as payloads for plants, including their applicability, benefits, and drawbacks, in this paper. The unique optical and electrical features of these nanoscale semiconductor particles represent a fresh strategy for boosting agricultural output and plant health. Precision farming, in which technology plays a central role in maximising resource utilisation and crop management, has become increasingly popular in recent years. Because of their distinctive properties, hybrid quantum dots are among the most intriguing options to aid in this transition. Let's dig into the reasons why they're so promising. Hybrid quantum dots, first and foremost, have extraordinary potential for nutrition monitoring in plants. These nanoparticles can be modified to bond with nutrients like nitrogen, phosphorus, and potassium by being equipped with the appropriate receptors. With this development, nutrient levels in plant tissues may be tracked in real time. With this level of specificity, farmers can adjust their fertilisation practises to be more efficient and gentler on the environment. The detection of plant diseases and pests is another intriguing use of hybrid quantum dots in agriculture. These nanoparticles may function as early warning systems if sensors capable of recognising particular infections or insect pests were attached to them. Quantum dots can alert farmers to the presence of a threat while a plant is under attack. By taking preventative measures, farmers can lessen the amount of chemical pesticides they use, which is good for the environment and the economy. Additionally, hybrid quantum dots can be used as supplemental artificial light sources for plant photosynthesis. Quantum dots augment daily "light period" for plants by generating light in the photosynthetically active radiation (PAR) spectrum, which is not found in natural sunlight. As a result, plant growth and productivity are increased even in areas with less sunshine or during seasons with shorter daylight hours. The benefits of hybrid quantum dots in the agricultural supply chain extend beyond their role in plant health and growth. It is now feasible to track the origin of agricultural goods with unprecedented accuracy thanks to the use of unique quantum dot identifiers applied during the growing cycle of a plant. In addition, the markers allow for constant quality check, guaranteeing that goods continue to be authentic and up to par as they make

their way through the supply chain. Finally, the promising new field of contemporary agriculture is the use of hybrid quantum dots as payloads for plants. Crop yields, environmental impact, and the efficiency and sustainability of agricultural practises can all benefit from their abilities to monitor nutrients, identify pests and diseases, boost photosynthesis, and provide traceability. However, in order to fully utilise the potential of hybrid quantum dots in agriculture, it is crucial to overcome the obstacles connected with their deployment, such as potential toxicity and environmental effects.

2 Different Types of Nanocarriers for Plants

Improved agricultural productivity and pathogen resistance are only two examples of the many uses for nanomaterials (NCs) such metal NPs, carbon-based materials (CNTs, CNFs, graphene, and graphene oxide), polymers, and lipids. At larger concentrations, however, these NCs can have toxic effects on the plant. In most cases, NCs result in much greater seed germination, root/shoot length, floral output, and fruit yield.

The delivery of agrochemicals makes considerable use of numerous metal-based NCs (including Cu, Zn, and Ag) and metal oxides (including TiO_2, CuO, ZnO, and AgO). In addition, most metal-based NCs are incorporated into plant tissues as micronutrients, promoting healthier plant development [17]. To measure NP accumulation and plant growth, for instance, researchers utilise Cu-NPs in mung (Phaseolus radiatus) and wheat (Triticum aestivum). The results indicated that increased concentrations of Cu metal ions within the plants led to slower growth in young seedlings. The accumulation rate is proportional to the amount of Cu that plants are subjected to [18]. The effects of selenium (Se) and copper (Cu) nanoparticles (NPs) on tomato plants were studied by Hernández et al. It appeared that the antioxidant capacity of tomato fruits was enhanced by both NPs. The concentration of Se in the Se-NPs boosted the yield of tomatoes. Tomato yield was likewise boosted, albeit at a lower concentration, when Cu-NPs were used. Tomato yield is not affected by the concentration of Cu-NPs [19]. There have been a number of studies indicating that using metal-NPs in plant growth is beneficial. These metal NPs improve plant growth by boosting the metal NP concentration. However, phytotoxicity may be induced by metal NPs at larger concentrations. Metal NPs, in general, served as both micronutrients and NCs, boosting plant development.

Activated carbon, activated carbon nanofibers (ACFs), carbon beads, and carbon-based nanomaterials like graphene, graphene oxide, carbon nanotubes (CNTs), and carbon nanofibers (CNFs) are just some of the many carbon-based materials that find widespread use in fields as diverse as energy, environment, medicine, sensors, and agriculture [20]. Plants make effective use of these carbon-based NCs, which are employed to stimulate growth. Translocation within plants increases growth, and it is interesting to note that CNTs and CNFs can penetrate the seed coat [21]. Tomato seeds were used as a test subject for graphene. Graphene was found to improve a

plant's germination rate, root length, and shoot length, according to the data [22]. Graphene quantum dots (G-QDs) were synthesised by Chakravarty et al. and tested on coriander and garlic plants. The results indicated that G-QDs acted as growth stimulants, aiding in the development of the plant's leaves, roots, shoots, flowers, and fruits [23]. Wheat (*Triticum aestivum L.*) has also been shown to respond to graphene in a separate study. The results indicated that graphene promoted root growth in wheat plants but had no effect on leaf development. Graphene exposure also impairs photosynthesis and biomass production by damaging roots and causing oxidative stress. In addition, the research indicated that the *Triticum aestivum L.* plant may have negative consequences from prolonged exposure [24]. Graphene oxide (GO) is a graphene derivative that can be easily functionalized with a surface functional group that has great biocompatibility, leading to its widespread use in a wide range of end applications. For instance, Hu et al. studied how GO interacted with plant cells. Inhibition of cell development, production of reactive oxygen species (ROS), and disruption of enzymatic function were all observed, suggesting that GO translocates easily within cells that are damaging to plant cells [25]. Researchers are continually interested in CNTs for a wide range of uses due to their exceptional physicochemical properties, including medication delivery, energy, environment, sensing, and agriculture. Cu/Zn-CNFs were also synthesised by the same set of researchers, who likewise integrated them into a polymeric (PVA-starch) matrix. The results indicated that the soil application of the developed Cu/Zn-CNF-PVA-starch-based formulation promoted greater chickpea plant development. In comparison to Cu/Zn-CNFs, the polymeric formulation's controlled-release of CNFs and Cu/Zn micronutrients is a benefit. The biodegradability of the Cu/Zn-CNF-PVA-starch formulation was also enhanced by the inclusion of starch. A related group also synthesised a bacterial homoserine lactone with Fe-CNF incorporation for use against the chickpea plant. Fe-CNFs were found to be effective in transporting bacterial homoserine lactone, which promotes plant growth and provides resistance to pathogens [26]. The same team also looked at the usage of Cu-CNFs to scavenge chromium while absorbing nutrients. In spite of Cr contamination, the data revealed that the Cu-CNFs improved water absorption capability [27]. Research has shown that CNFs can improve plant growth by acting as NCs in the delivery of micronutrients, increasing water intake, accelerating seed germination, and lengthening the plants' roots and shoots.

Applications ranging from medication administration to transdermal patches to wound healing to agriculture rely heavily on a wide variety of polymers such polyvinyl alcohol (PVA), starch, polylactic acid (PLA), poly (lactic-co-glycolic acid) (PLGA), hyaluronic acid (HA), gelatin, and chitosan [28]. These polymers were able to efficiently transport fertilisers and micronutrients inside plant tissues. These polymers are typically encapsulated within the micronutrients, fertilisers, or metal NPs, changing the surface charge of the materials and allowing for effective translocation within plants and controlled release of micronutrients. Deshpande et al., for instance, synthesised Zn-loaded chitosan NPs and evaluated their efficacy as a plant micronutrient. The results indicated that Zn-loaded chitosan NPs could be an effective vehicle for transporting micronutrients [29]. Synthesis of gibberellin phytohormone-containing nanoalginate-chitosan and nanochitosan-alginate composites was the

topic of a separate study. The results indicated that polymeric NCs successfully release the growth-promoting phytohormones with no negative side effects on the plants [30].

As research into nanotechnology has progressed, it has become increasingly applicable in the industrial sector. The primary goal of a controlled-release delivery system in agricultural and other biological applications is to provide crops with the necessary fertilisers, agrochemicals, and micronutrients over an extended period of time while reducing their exposure to toxic levels. The NCs' readily functionalizable nature, large surface area, and quick mass transfer make them ideal for the efficient administration of fertilisers, agrochemicals, and micronutrients into plants. From this vantage point, various NCs (materials with dimensions of 100 nm or less) are introduced into fertilisers, agrochemicals, and micronutrients to facilitate their entry into plant cells. Several different approaches are taken to include NCs in fertilisers: Agrochemicals can be attached to NCs in four different ways: (1) encapsulation, in which fertilisers are encased on the surface of NCs; (2) absorption, in which NCs are absorbed onto fertilisers or fertilisers are absorbed onto NCs; (3) attachments through bonds; and (4) entrapment. The integration procedure contributes to the development of a reliable delivery system that allows for the measured dispersal of agrochemicals. In addition, NCs improve the stability of fertilisers, agrochemicals, and micronutrients, protecting them from degradation and leaching out into ecosystems and guaranteeing good efficiency even at low concentrations of agrochemicals and reduced toxicity. The size and surface charge of the NCs are the two most important elements in NC delivery within plants. Translocation of NCs (or micronutrients) within plants is influenced in part by their surface charge. While NCs can be either positively or negatively charged, plant cell walls are typically negative in charge. Higher buildup and reduced translocation of the positively charged NCs occurs due to positive–negative interactions. There may be negative consequences on plant growth from the increased accumulation. The repulsion forces between the negatively charged NCs and the positively charged plant components allow for efficient translocation of the NCs from root to shoot to leaf. Increased NC translocation ability benefits plant development [31]. The magnitude of NCs is, like surface charges, a crucial element. Translocation of NCs or nanomaterials up to 500 m is straightforward within plants. There are two main routes that NCs and other nanomaterials take: (1) apoplastic, which involves transport through the xylem, cell walls, and extracellular space, and (2) symplastic, which involves movement through the cytoplasm of neighbouring cells or plasmodesmata. About 50 nm-sized particles use symplastic pathways, while NCs larger than that use apoplastic pathways to move around inside plants.

3 Uptake, Translocation and Accumulation of CDs in Plants

QDs' distinctive fluorescence signal offers a useful method for tracking them in plants. Using fluorescence imaging, transmission electron microscopy (TEM) observations, or Raman spectroscopy, researchers have seen plants take in QDs [32] (Fig. 1). For instance, Loukanov et al. studied CD uptake in live tobacco bright yellow-2 (BY-2) plant cells using fluorescence and confocal imaging [33]. For our prior research into plant CD uptake and translocation, we used the mung bean as a model plant [34]. The CDs are made readily available for absorption and uptake by the roots of the mung bean plants thanks to hydroponics. The mung bean seeds germinated and grew rapidly after 5 days of cultivation at 25 °C. Increased absorption of CDs by the mung bean plant was demonstrated by the evident concentration-dependent reddish-orange fluorescence under 365 nm UV light (Fig. 2a). After 5 days of incubation, the mung bean seedlings were analysed for CDs by taking images of their roots, stems, cotyledons, and leaves with a confocal laser scanning microscope. In Fig. 2b and c, we notice that the fluorescence signals of the CDs are mostly localised in the vascular system of the root, stem, and leaves of the mung bean sprouts. The CDs were agglomerated and formed considerably bigger clusters in the intercellular space, as seen in TEM images of cross sections of control and CD-treated mung bean seedlings (Fig. 2d). In order to hasten the germination process, the CDs must first break through the seed coat via the intercellular space and then aggregate in the cotyledons. The CDs were absorbed by the root's surface when it emerged and then moved into the root's vascular bundles. The CDs were then transported via the vascular system from the roots to the stems and leaves, where they settled in the veins rather than the mesophyll system (Fig. 2e). Kang et al. observed a similar mechanism for the upward transport of CDs from the mung bean root to the stem and leaves [35]. It is important to note that the surface modification of plants determines the pattern of CD distribution in those plants [36].

Genetic engineering is crucial to plant research. Due to cell wall inhibition, current delivery methods like Agrobacterium-mediated gene transfer and gene gun rapid gene expression system are difficult or limited in host range. Using both plant and animal cells, as well as whole plants, Wang et al. proved the efficacy of a nanocomposite (CDP) developed through mixing polyethyleneimine (PEI) with carbon dot (CD) as a vector for gene transfection [37]. Wheat (Triticum aestivum), mung bean (Phaseolus radiatus), and rice (Oryza Sativa japonica) leaves and rice roots are all capable of efficient DNA delivery and robust gene expression without DNA integration (Fig. 2). Notably, the CDP induced hygromycin resistance in rice by successfully delivering the hydamycin resistance gene into the rice. Furthermore, CDP is capable of delivering and expressing the -glucuronidase gene in mature rice embryo generated callus. In addition to easing plasmid entry into cells, the CDP safeguards DNA from degradation by DNase. Our research yields a very effective DNA delivery system for rapid gene expression, which may prove to be a time-saving and beneficial gene engineering tool for studies in plant physiology.

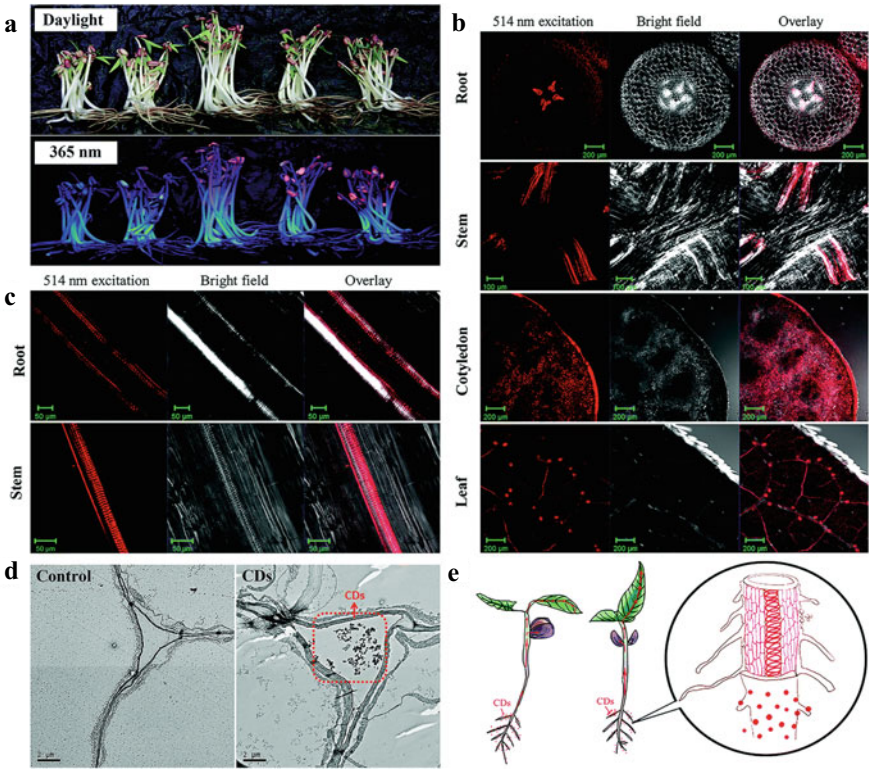

Fig. 1 CD uptake and translocation in mung beans. **a**–**e** Mung bean sprouts under daylight and 365 nm UV light; LSM images of root, stem, cotyledon, and leaf (**b**) and longitudinal sections (**c**) cultured with CDs (1.0 mg mL − 1) for 5 days; TEM image of roots cultured with pure water and CDs (1.0 mg mL − 1) after 5 days; and schematic illustration of CD uptake, transport, and accumulation. Reproduced with the permission from ref. [34] © 2016 American Chemical Society

4 QDs Delivery in Plants

The capacity to engineer plant function is hampered by the fact that current methods for nanomaterial delivery in plants lack the precision necessary to target specific subcellular compartments. One study developed a nanoscale platform that directs the delivery of nanomaterials containing biochemicals to the chloroplasts of plants, the cells' photosynthetic organelles [38]. Confocal microscopy imaging and quantitative detection by elemental analysis are both made possible by the fluorescence emission of quantum dots (QDs) in a low background window. Nanoparticle coating with a rationally chosen and conserved guiding peptide focuses their distribution to chloroplasts, while QD functionalization with -cyclodextrin molecular baskets enables loading and delivery of different drugs. In plant cells in vivo, peptide biorecognition allows for very efficient and precise delivery of QD with chemical cargos to chloroplasts (74.6 10.8%) and more finely tunable alterations in chloroplast redox activity

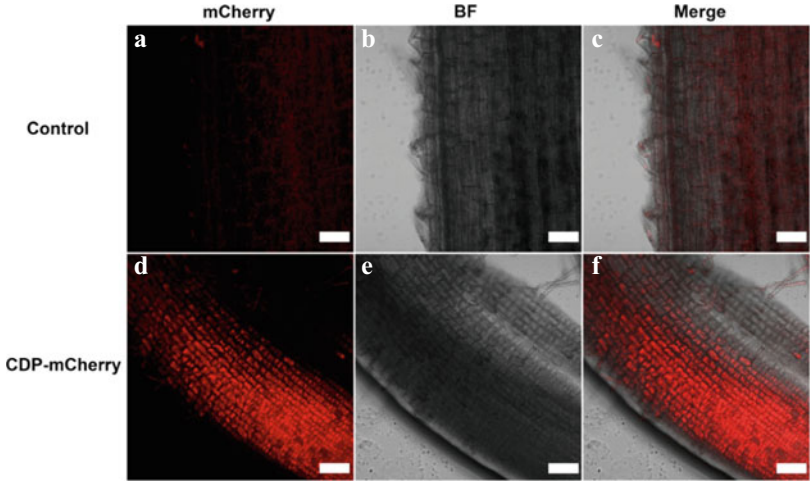

Fig. 2 CDP gene delivery and transient mCherry expression in rice root integrity. **a–c** Rice root was treated with 10 ng/μL pJIT163-mCherry plasmid without CDP. **d–f** Rice root treated with 10 ng/μL CDP-pJIT163-mCherry plasmid. The majority of cells showed red fluorescence (scale bar: 40 μm). Fluorescence confocal micrographs were taken after 12 h of soaking. Reproduced with the permission from ref. [37] © 2020 American Chemical Society

than can be achieved with chemicals alone. Applications of nanotechnology in plant biology, bioengineering, nanoparticle-plant interactions, and nano-enabled agriculture abound due to the precision with which chemical payloads can be delivered in the form of nanomaterials using biorecognition patterns. To research, augment, or impart unique plant functionalities, plant nanobiotechnology is an interdisciplinary area at the intersection of nanotechnology and plant biology. Nanoparticle interactions with plants and the effect of nanoparticles on plant function need the first step of delivering nanoparticles to plants in vivo. The uptake, transport, and dispersion of nanoparticles in plants can be studied with readily available confocal imaging equipment since quantum dots are smaller than plant cell wall pores, have variable surface chemistry, strong fluorescence, and do not photobleach. Another work revealed three approaches to transporting quantum dots into the leaves of living plants: lamina infiltration, entire shoot vacuum infiltration, and translocation from the roots to the leaves. Nanosensors and nanoparticles used in medicine administration are two examples of nanoparticles that could benefit from these techniques [39]. Plants are a key link in the bioaccumulation of nanoparticles into the food web and a possible route for their transfer to the environment. Currently, scientists are conducting research to determine how various nanoparticles affect the development and metabolism of plants. The plant's cell wall prevents outside substances, such as nanoparticles, from entering the cell easily. Pore diameter of cell wall varies from 5 to 20 nm, which establishes sieving properties [40]. This means that only nanoparticles or nanoparticle aggregates with a diameter smaller than the pore diameter of the cell wall can easily go through to the plasma membrane [41]. In addition, tailored nanoparticles can interact with cell walls to

either enlarge preexisting pores or induce new pores, both of which boost nanoparticle uptake. During endocytosis, the plasma membrane forms a cavity-like shape around the nanoparticles, facilitating their further internalisation. It is also possible for them to pass across the membrane via in-membrane transport carrier proteins or ion channels. It is possible that the nanoparticles connect with various cytoplasmic organelles and disrupt metabolic activities there [42]. When nanoparticles build up on a plant's photosynthetic surface, they increase the temperature of the leaves, which in turn alters gas exchange via stomatal blockage and has knock-on effects on a number of other physiological and cellular processes [43]. The utilisation of plants as a source for nanoparticle synthesis has been the subject of additional research following the discovery of the mechanism of nanoparticle uptake and creation within plants.

An efficient aqueous method for preparing biocompatible QDs for monitoring nanoparticle-mediated gene transfer in conjunction with Jatropha curcas molecular breeding has been devised. Self-assembly was used to create water-soluble CdSe nanoparticles using L-Cysteine as a stabiliser, and an optimal synthesis technique was determined with the help of fluorescence spectroscopy. The QDs were utilised to electrostatically label chitosan-DNA nanoparticles, and the resulting QD-labeled chitosan-DNA complexes exhibited enhanced fluorescence qualities, as seen by a red shift in the emission and absorption spectra compared to those of the CdSe QDs alone. Instead of using Agrobacterium to induce genetic transformation in Jatropha curcas, this approach is being investigated as a potential improvement. Transfection of Jatropha curcas cells using CdSe/CS-DNA complexes does not appear to have resulted in DNA digestion, as evidenced by PCR amplification of the entire length of the carried reporter gene (GFP). In addition, fluorescence detection of the GFP gene indicates that the target DNA was integrated into the plant genome in the transfected callus cells [44]. In a separate investigation, researchers presented the new idea of producing quantum dots using plant materials. The aqueous extract of the root of the plant Rubia cardifolia was used to synthesise the copper nanoparticles in the form of quantum dots. SEM, TEM, AfM, and fluorescence microscopy were all used to characterise the produced quantum dots. The 22.68 nm quantum dots (QDs) synthesised were spherical particles with rough surfaces. The use of AfM demonstrated the green fluorescence characteristic of these quantum dots. The antimicrobial properties of Cu quantum dots were also examined. CuQDs exhibited strong antibacterial activity against *Klebsiella pneumoniae*, *Shigella spp.*, *Escherichia coli*, *Plesiomonas shigelloides*, *Streptococcus aureus*, and *Pseudomonas aeruginosa*, as measured by colony forming capability testing and diameter of inhibitory zone measurement [45]. In order to determine how CdS-QDs compare to Cd^{2+} ions and bulk-CdS in terms of bioaccumulation and biological response, they were applied to soybean seedlings at concentrations ranging from 50 to 200 mg/L. In order to learn how different polarity and surface charge ligands affect QD stability, uptake, translocation, subcellular localization, and cellular response, bare CdS-QDs were coated with them [46]. QD-TOPO, QD-PVP, QD-MAA, and QD-GLY were all synthesised using four different ligands: trioctylphosphine oxide, polyvinylpyrrolidone, mercaptoacetic acid, and glycine. QD-MAA particles were the most stable, remaining 332 nm in size with

minimal Cd^{2+} dissolution (9%) after 14 days in aqueous suspension, while QD-TOPO produced huge aggregates of 3861 nm in size with 27% Cd^{2+} dissolution. Soybean roots collected Cd at a rate of 568 (QD-MAA) to 1010 (QD-PVP) g/g tissue dry weight (DW) after being exposed to 100 mg/L bare and coated CdS-QDs in vermiculite for 14 days; this was statistically equal to the bulk-CdS treatment (639 g/g DW). QD-TOPO accumulated mostly in the membranes, while Cd from $CdCl_2$, bulk-CdS, QD-MAA, and QD-GLY accumulated primarily in the cell wall (40–55%) followed by organelles (28–40%), suggesting apoplastic route. The exception was QD-PVP, which translocated Cd from the roots to the shoots, sequestered it primarily in organelles (49%), and hence lowered leaf biomass. Peroxidases were found to have a crucial function in mitigating the oxidative stress that was induced by CdS-QD exposure. Root lignification permitted the plants to limit Cd accumulation even at the highest CdS-QD treatment (200 mg/L), with the exception of QD-PVP, where lignification was reduced by 21%, resulting in increased Cd content in the shoots. The plants that were subjected to 200 mg/L QD treatments exhibited stress resistance by increasing the amino acid content of their leaves. This research demonstrates how surface coating significantly affects the fate and impacts of QDs in a planted system.

Plants produce phytochemicals like melatonin and serotonin to help them adapt to adverse conditions. Although their synthesis pathways and enzymes have been extensively studied, and a phytomelatonin receptor was recently identified, the actual molecules themselves remain elusive. Understanding where indolamines are found in biological tissues can be a useful resource for studying their role in organisms of all kinds. Quantum dot nanoparticles were used in this investigation for the first time to enable direct visualisation of melatonin and serotonin in axenic roots, yielding exciting new insights. Epidermal cells took up melatonin, transported it laterally, and it eventually built up in endodermal and rapidly dividing pericycle cells [47]. There was a rapid polar migration of serotonin-absorbing cells from the crown region to the root tip. Melatonin and serotonin lost their localisation and were disseminated throughout cells as a result of thermal stress. These results show that melatonin and serotonin travel naturally in the root system to guide cell proliferation and imply that plants have a system to distribute the indolamines throughout tissues as antioxidants in response to environmental challenges. The field of biomedicine is looking to quantum dots (QDs) as a potentially useful tool [48]. They could be used in bio-labels and devices that aid in genetic manipulation and post-harvest management in the agricultural sector, as well as in the production of innovative pesticide formulations. We studied the cytotoxicity and genotoxicity of CdSe QDs in a model plant (Allium cepa) and identified connections between QDs' genotoxic activity and oxidative stress since interactions with higher plants are of significant environmental and ecological importance. Three different concentrations of CdSe QDs (12.5, 25, and 50 nM) were applied to Allium cepa bulbs with healthy roots. For cytotoxicity, we looked at cell viability and mitotic rates; for genotoxicity, we checked for DNA lesions, chromosome abnormalities, and micronuclei. We found that QDs caused oxidative stress and genotoxicity in a considerable way. The maximum uptake of CdSe QD occurred at 50 nM, which may be connected to the fact that Cd is retained

in Allium roots in a dose-dependent manner. Both superoxide dismutase (SOD) and catalase (CAT) and glutathione peroxidase (GSH) were triggered in response to the oxidative stress caused by CdSe QD treatment. CdSe QDs were reported to be cytotoxic at 25 nM and genotoxic at 50 nM in a plant study. These results pave the way for figuring out what concentrations to utilise when putting these nanodevices to use on plants in the real world. CdTe quantum dots (CdTe-QDs) are a popular type of NP, and their influence on wheat seedlings when combined with ultraviolet B (UV-B) light was studied by Chen et al. The physical features of 5-day-old seedlings were used to test various concentrations of CdTe-QDs and UV-B radiation, both separately and in combination. Wheat antioxidant enzymes were activated when seedlings were treated with either CdTe-QDs (200 mg/L) or UV-B radiation (10 kJ/m^2/d). DNA laddering revealed that the buildup of CdTe-QDs in plant root cells triggered programmed cell death. Both CdTe-QDs and UV-B radiation were found to reduce root and shoot development [49]. Combination therapy appeared to have an additive inhibitory impact. The impact of UV-B and CdTe-QDs on plant development was detailed in this study. The discovery that CdTe-QDs accumulate in plants over the course of their lives emphasises the importance of continuing to monitor these connections.

The overspray of manmade nanoparticles into agricultural land is a major concern to food safety. Although there is great heterogeneity amongst rice cultivars in terms of cadmium accumulation, nothing is known about their reactions to Cd-based nanoparticles [50]. Two rice cultivars with varying Cd accumulation capability were studied for their ability to accumulate cadmium telluride quantum dots (CdTe QDs at 0.5, 1.0, 2.5, and 5.0 mg-Cd/L). After 7 days, the high-Cd-accumulating variety (T705) exposed to 5.0 mg-Cd/L of CdTe QDs showed minimal growth suppression in comparison to the low-Cd-accumulating variety (X24). Cd levels in the roots of both rice cultivars were similar, however T705 had a greater Cd level in its shoots than X24. The accumulation and transfer of CdTe QDs from roots to shoots in T705 has been seen using transmission electron and confocal laser scanning microscopy. T705 leaf activities and gene expression of antioxidative enzymes rose more than X24 leaf activities and gene expression. Our research is the first to confirm that various rice types accumulate Cd differently when exposed to Cd-based QDs, and that this variation has a genetic basis that needs to be explored further.

5 Challenges and Future Perspectives

QDs, or quantum dots, are nanoscale semiconductor materials that have garnered significant interest in many disciplines—from biology and agriculture to physics and chemistry. Scientists have recently investigated the use of quantum dots in plant studies, with the goal of better comprehending plant absorption mechanisms and developing more effective agricultural practises. Although there is much to be gained from incorporating nanoparticles into plant science, there are also several obstacles that must be overcome before they can be used safely and effectively. Quantum

dots present a number of hurdles for plant researchers due to the complexity of the systems involved in their uptake and dispersion throughout plant tissues. Scientists have made great strides in following quantum dots as they go from the soil to the roots of plants and then to the various organs of those plants. But the fundamental mechanisms are still poorly understood. Root absorption and foliar application are two methods by which quantum dots can penetrate plants. How they get from one place to another inside the plant, like across cell membranes and through the vascular system, are still a mystery. Understanding these mechanisms is crucial for weighing the costs and benefits of using quantum dots in fields like agriculture and environmental monitoring. The potential toxicity of quantum dots to plant life and ecosystems is another major challenge linked with quantum dot research on plants. Heavy metals like those found in quantum dots, such as cadmium, lead, or selenium, are deadly to living things when ingested in sufficient quantities. The potential of environmental contamination cannot be completely ruled out, despite efforts to build less hazardous or non-toxic quantum dots. To ensure that the use of quantum dots in agriculture does not affect plant health or disturb ecosystems, researchers must conduct rigorous toxicity analyses. In order to safely use quantum dots, it is essential to understand the threshold concentrations at which they become damaging to plants. Quantum dots have the potential to revolutionise agriculture, but overcoming issues of scalability and cost-effectiveness are necessary for their broad use. Large-scale manufacturing and deployment of quantum dots is now cost prohibitive in many agricultural settings due to the high cost and resource requirements of current techniques of synthesis. There are current efforts to provide greener, more economical ways to produce quantum dots. If this works, it might cut down on production costs and open the door to widespread use in farming. The application of quantum dots in plant research and agriculture necessitates careful consideration of regulatory and ethical considerations, as is the case with any new technology. It is crucial to make sure these nanoparticles are employed in a way that doesn't endanger people or the environment. The novel difficulties presented by quantum dots in farming necessitate the creation or modification of regulatory structures. This includes creating disposal and waste management procedures, monitoring their use for safety, and setting standards for use.

The investigation of quantum dots in plants has the potential to significantly advance the fields of agriculture and environmental monitoring. Uptake mechanisms, potential toxicity issues, enhancing scalability, and establishing regulatory frameworks are just some of the obstacles that researchers and stakeholders must overcome. If these challenges can be surmounted, quantum dots have the potential to become useful instruments for improving crop management, gaining insight into plant physiology, and increasing sustainable agriculture. To ensure that agriculture and the environment benefit from and are not endangered by the use of this technology, it must be approached with caution.

6 Summary

At the crossroads of nanotechnology and agriculture, hybrid quantum dots are an exciting new development with the potential to significantly impact farming and environmental monitoring. These hybrid nanoparticles, which combine the biological components with the amazing optical and electrical capabilities of quantum dots, have the potential to revolutionise our understanding of and interaction with plant life. Hybrid quantum dots can be used as payloads for plants, allowing for highly precise monitoring and manipulation of many aspects of plant life. Hybrid quantum dots' capacity to deliver real-time insights on plant health and physiological processes is a major benefit of their use in agriculture. These tiny sensors may be programmed to detect a wide range of environmental and biological cues, enabling for real-time tracking of situations including nutrient availability, water stress, and disease risk. Such monitoring enables farmers and researchers to improve resource utilisation, react quickly to threats to crop health, and make data-driven decisions. Hybrid quantum dots may also play a pivotal role in the future of crop nutrition control. By modifying these nanoparticles to slowly release vital nutrients, we can give plants exactly what they need, when they need it. This specific method not only increases crop productivity but also reduces the environmental damage caused by runoff fertilisers, a major issue in today's farming. Hybrid quantum dots also have use in environmental monitoring. These nanoparticles can be used to monitor the movement of pollutants and heavy metals in water and soil, revealing important information about the state of ecosystems. Their ability to detect and mitigate environmental problems early on is a major factor in their value in protecting biodiversity and ensuring agriculture's long-term viability. Yet there are obstacles to overcome before hybrid quantum dots can be widely used in farming. First and foremost is making sure these nanoparticles don't hurt anyone or the planet too much. Guidelines for safe usage and reduction of potential toxicity should be prioritised. Scalability and cost-effectiveness are other important considerations for making this technology widely available to farmers and scientists. In conclusion, using hybrid quantum dots as payloads for plants is a forward-thinking strategy for dealing with the complex problems facing modern agriculture and environmental protection in this day and age. They are a game-changer in the agricultural sector because of their capacity to provide real-time monitoring, improve nutrient management, and contribute to environmental sustainability. While there are still obstacles to overcome, the potential gains in crop yields, resource efficiency, and environmental protection offered by this technology are too great to be disregarded. Hybrid quantum dots have the potential to usher in a new era of intelligent, environmentally friendly farming if current research and development efforts are successful.

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Quantum Dots in Light Emitting Diode and Their Applications

Nallin Sharma and Chandan Srivastava

Abstract The development of confined dimensionality in nanoscopic materials has paved the path for many promising applications for human societies. One aspect is zero-dimensional quantum dots (QD) synthesized by various methods. These QDs have a significant role in technological advancements of various healthcare and environmental applications. In this current section, methodologies involved in fabricating quantum dot light emitting diode (QLED) are discussed. The phenomenon of emitting light from provided electrical energy is termed electroluminescence (EL). The mechanisms involved in obtaining electroluminescence are vital to build a working understanding. Three major mechanisms namely, energy transfer, field inducing, charge injection is prominently discussed in the coming sections. Furthermore, the advancements made in developing QLEDs are of huge interest, detailed of type I, II, and III structures are provided. Eventually the day-to-day modern applications of QLEDs in data communication technologies, sensor technologies, and data display technologies are covered with wider information range.

Keywords Quantum dots · Quantum light emitting diode · Color rendering index (CRI) · Luminous efficacy (LE) · Light fidelity (Li-Fi) · Augmented reality (AR) · Virtual reality (VR) · Temperature sensors

1 Introduction

In the ever-developing world scenario, a need for outperforming technologies is everlasting. Devoting a group of nanomaterials in Light emitting diodes have resulted in many outperforming applications. Quantum Dots (QD) as discussed in earlier sections are zero-dimensional confined nanostructures possessing atomically precise

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orientation capabilities. Utilizing QDs in fabricating efficient devices is one approach that evolved many research interests into futuristic technologies. One remarkable possibility is by developing optically tunable light emitting diodes. These Quantum dot light emitting diode (QLED) have replaced high power lasers in multiple arenas. QLEDs have grown to market capturing technology of our present age. The overwhelming performance of QLEDs has been a result of continuous and rigorous research work performed by the scientific community. The assembling of QLEDs is one prominent area of study that in general deals with enhancement of quantum yield (QY), lifetime, and luminance of overall devices. Incorporating QDs in light emitting diode (LED) was a breakthrough of its time that paved the way to zenith of modern technology.

Many synthesis approaches were reported as discussed in earlier sections, here the emphasis is more towards QLED designing, architecture, luminance mechanism, and applications. As the theory of semiconductor engineering begins with p–n junction diodes, similarly LEDs have assembling of many layers to promote electron transfer and recombination at the end of its journey. The desired product of the whole process is generation of visible light or photons having certain wavelengths. These generated photons have energy which is eventually either directly utilized or transferred in another form of operational entity. Owing to QDs size and orientation orders, marvelously engineered products can be seen in day-to-day life. Precisely small sized QDs brighten scenarios of complex device architecture with an ease of operations, functional components thus undergo comparatively lesser frictional, tribological, mechanical, and optical losses. QLEDs thus have been an astounding candidate of nanomaterial engineering to demonstrate miraculous scientific and technological achievements. The new age displays, data communication, sensor technology, and many others are vitally based on QLEDs.

Here in the following section, details of QLED functioning and evolving developments in various applied research are discussed with utmost interest to their fabrication developments till date. The photoluminescence quantum yield (PLQY) is one critical aspect to measure the efficiency of QLEDs and various other factors such as color rendering index (CRI), color quality scale (CQS) are discussed in detail. The later sections of the chapter encompass modern applications of QLEDs in data communication, sensors, and various data display technologies.

1.1 Color Scheme

Chromaticity is a fundamental unit used to define the visible wavelength ranges being emitted from any photon source. Scientifically, the range is defined by tristimulus coordinate system (x, y) that signifies color code as perceived by human vision. The coordinate system comprises of three axes namely x, y, and z but the dependence of z $= 1 - x - y$; delivers complete information with-in two axes i.e., x and y. Conventional chromaticity database is represented by standards of Commission Internationale d l'Eclairage, the international commission for illumination [1, 2]. The Commission

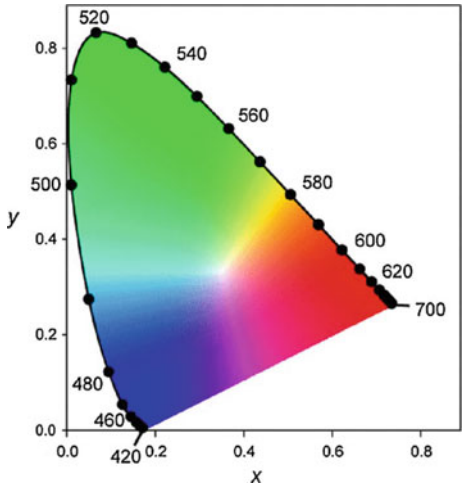

Fig. 1 Two-dimensional representation of standard CIE-1931 chromaticity diagram. (inspired from citation [3])

Internationale d l'Eclairage society has various charting schemes for representing 32 million deep focused colors, CIE-1931 is one standard widely acknowledged for defining visible chromaticity from LEDs (Fig. 1).

Every tristimulus value represents color codes in terms of basic three colors Red (R), Green (G), Blue (B) color, where $\overline{x}(\lambda)$, $\overline{y}(\lambda)$, and $\overline{z}(\lambda)$ are color match function and P(λ) is power density.

$$X = \int_{\lambda} \overline{x}(\lambda) P(\lambda) d\lambda$$

$$Y = \int_{\lambda} \overline{y}(\lambda) P(\lambda) d\lambda$$

$$Z = \int_{\lambda} \overline{z}(\lambda) P(\lambda) d\lambda$$

The equal energy locus located in the center of the chromaticity at (x, y, z) = ($\frac{1}{3}$, $\frac{1}{3}$, $\frac{1}{3}$), yet this ideal point is not the observed locus for each observation. Hence, the values are comprehended to (x, y) coordinates.

1.2 Color Temperature and Color Rendering Index

Black body radiation paves the basic understanding of color temperature, deduced from the Planckian locus plotted on standard CIE-1931 representation in x and y coordinate system. The color temperature of light source is determined with respect to black body radiation temperature in Kelvin (K) [1] (Fig. 2).

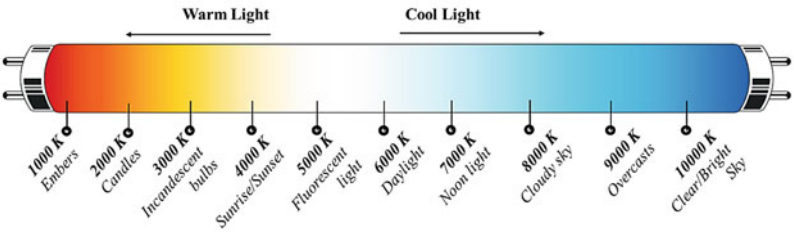

Fig. 2 Schematic representation of color temperatures with respect to observed natural illuminations. (Inspired from citation [3])

In many cases, color temperature does not fall in Planckian locus plot, and a corelated color temperature (CCT) system is used. The (x, y) coordinate system is converted to (*u'*, *v'*) system using the equation mentioned below. CIE-1976 was introduced to simplify these complications, and standard plot with (*u'*, *v'*) coordinates along the respective color temperatures could be defined. The coordinates system was derived using mathematical equation as stated below:

$$u' = \frac{4x}{-2x + 12y + 3}$$

$$v' = \frac{9y}{-2x + 12y + 3}$$

Capability of light source to reveal true color of illuminated object is termed as Color rendering index (CRI) [4]. The measure scale is between -100 to 100, where the best score is determined by 100 and vice versa. CRI measuring of perfect illumination source is relative to 100% blackbody radiation, maintained relative to measure color rendering index of usual illumination sources. Typical household illumination sources are above 80 CRI score, so that human vison is not tempered or harmed due to poor color rendering of objects illuminated. In cases particular of LEDs, color rendering of illuminated objects differs from blackbody standards and hence an enhanced scaling is observed for such case termed as Color Quality Scale (CQS). The new scale has gained tremendous impact over past few years to determine illumination quality of LEDs. CQS was developed by W. Davis and Y. Ohno in years 2004–05 [5, 6]. CQS implies 15 different reflective Munsell samples and records respective saturation factor of illuminated object that has gained intensified chroma, finally an enhanced information of colors is acquired depending upon illuminated saturation factor. This technique is effective against bright illuminating white light LEDs as compared to CRI; colors can be distinguished with percentage level of illumination under ambient conditions. CQS have amplified color rendering procedures in digital image processing techniques as well, by defining precise coloring score range under controlled illuminations [7]. In general, unlike CRI measuring the CQS technique scores between 0–100 that provides critical assessment by calculating root mean square (RMS) average of illuminated colors with respect to reference and

sample light source illumination. Groups of CQS values are now referred as standard for procuring ideal illumination conditions for any given area/volume, providing significant functional pioneering in lighting (luminous) devices.

Luminous efficacy is another prime concern for calibrating optimal or desired illumination intensity. Luminous efficacy of optical radiation (LER) and luminous efficiency of device (LE) are two distinct measurements performed to obtain lumen counts. An optical emission comprises of visible and in-visible radiations, visible are helpful in observing illuminated objects whereas, invisible radiations are source of infra-red (heat) or deep ultra-violet rays in some cases. The ratio of visible to invisible radiation or emissions helpful to human vision with respect to total emitted radiation is known as luminous efficacy of optical radiation (LER), measured in lumen per optical watt. On contrary luminous efficacy (LE) of device defines same ratio in terms of electrical power i.e., lumen per electrical watts. These are represented using mathematical equations as shown below:

$$LER = \left(683\frac{lm}{W_{opt}}\right)\frac{\int P_{opt}(\lambda)v(\lambda)d\lambda}{\int P_{opt}(\lambda)d\lambda}$$

$$LE = \left(683\frac{lm}{W_{opt}}\right)\frac{\int P_{opt}(\lambda)v(\lambda)d\lambda}{I \times V}$$

where, $P(\lambda)$ is spectral density signifying light power emitted per unit wavelength, $v(\lambda)$ is sensitivity function of human vision, I is current injection level and V is applied voltage level at operating point of LED.

2 QLEDs Structure and Mechanisms

The structure of QLEDs comprises of three major components namely, emitter, electron transport, hole transport. For the very primitive designs of QLED reported by *Colvin* et al. in 1994 [8], had Indium based transparent conduction oxide, QD-polymer based emitter, and base metal electrode. The direct use of metal electrode caused severe losses in overall device performance, yet a new era for light emitting sources was born. Next improvisation was made by Coe et al. by using organic charge transport layer for sandwiched QD emission layer and this kind of QLED fabrication technique is termed as type-I design. Eventually, inorganic charge transport layers were implied to enhance efficiency, Au/p-GaN/CdSe/n-GaN/In was overall design reported by *Mueller* et al. in 2005 and termed as type-II [9]. With considerable enhancement factor due to inorganic transport layer, next proposed type-III [10, 11] design had both charge transport layer of inorganic material being considered of par efficient till date. Many more improvisations are explored by the scientific community to address the size and efficiency challenges (Fig. 3).

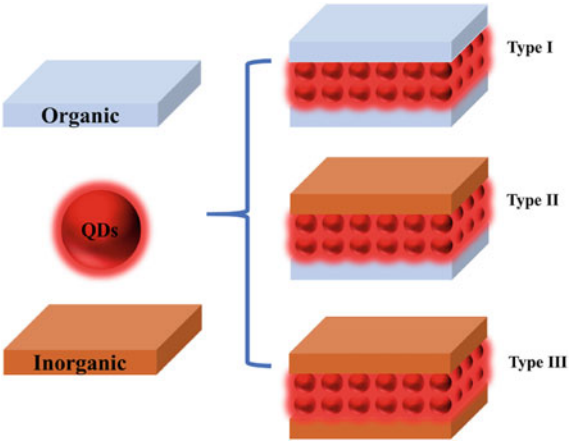

Fig. 3 General representation of QLED types. All inorganic as Type I, hybrid assembly as Type II, and all inorganic materials as Type III. (adapted from citation [12])

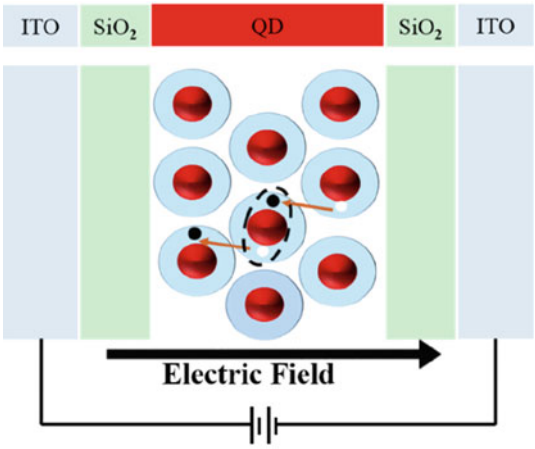

Fig. 4 Working mechanism of Field induced electroluminescence. (adapted from citation [12])

2.1 QLED-Electroluminescence Mechanism

Understanding the mechanism behind luminescence properties of QLEDs is crucial to build an efficient structure and fabrication process. Electroluminescence (EL) in general, is the process of releasing photons when excited electrons observe relaxation inhibition. For QLEDs, three major EL processes observed are namely, energy transfer, charge injection, and field induced.

2.1.1 Energy Transfer

The process where an excited donor generates electromagnetic fields in resonance to ground state acceptor, and later energy transfers to radiative localized state is termed as Förster resonance energy transfer (FRET). A sandwich QD layer present between the dipole acts as radiative emission layer and quantum confinement facilitates FRET in QD layer [13]. As QD are of 5×10^{-9} m scale and FRET is observed in a limited distance of $10–50 \times 10^{-9}$ m. This physical alignment has proved beneficial to enhance efficiency by controlling QD concentration to 7×10 [19] nanocrystals/L, the value further increases in solid-state assembling [14]. Apart from layer-to-layer energy transfer, inter-dot FRET or self-quenching phenomenon is also prominent in small packed QDs [15]. The surface-to-surface distance (D) in QD films is a beneficiary and limiting factor at same time, as it increases luminescence but on other hand degrades emission lifetime due to self-quenching.

2.1.2 Charge Injection

The process is considered crucial by many scientific researchers due to freedom of operations. Positioning of transport layers such that charge directly generates excitons in QD layer and radiative emission is observed, this phenomenon is termed as direct charge injection method [11, 16]. Sub-bandgap electroluminescence onset, formation of pseudo-band potential higher than threshold turn-on voltage capable of producing 0.1 cd/m [2] luminescence. It is essential to overcome various charge barriers or energy dissipation losses, termed as sub-bandgap turn-On phenomenon in charge injection QLEDs. The phenomenon was first suggested by Qian et al. and reported as Auger assisted energy up-conversion hole injection process in year 2010 [17]. Later an inversed transport layer structure was reported with exception direct charge injection due to electromagnetic coupling between QD layer and inorganic transport layer by Mashford et al. in 2013 [18]. This Auger assisted hole injection method achieved quantum efficiency of 90% by lowering driving voltage to sub-bandgap turn-On potential and gaining an overall 18% efficiency. Electrically pumped single-nanocrystal spectroscopy enlightens a conclusive remark to the long pursuing debate for charge injection phenomenon. Accumulating multiple scientific reports, a rational agreement is drawn that electron is injected first to charge neutral QD and thus forms a negatively charged intermediate (QD^-) resulting in formation of exciton state (QD^x). Hole injection is considered more demanding than electron injection to QD, supported by coulomb attracting effects. Once an electron injected to neutral QD state then hole injection potential barrier is lowered eventually, supporting QD to QD^- to QD^x to hole injection [19]. This strategy evidently limits multi carrier quenching effects due to presence of QD^- intermediate and inhibits over-hauling of charged electrons to QD layer causing Auger recombination. The feasibility of this method can be estimated by large scale production units agreeing the method as cost-effective.

2.1.3 Field Induced

As the name suggests, process of achieving radiative emission from QD layer sandwiched between two insulating or wide bandgap transparent conducting oxide is termed as field induced emission in QLEDs. The applied electric field across two oxide layer acts as charge transporter for sandwich QD layer, obeying coulomb attraction phenomenon [20]. In field induced emission, QDs are subjected to ionization due to induced potential from one charge transport layer, the ionization then leads to forming a hole and forces ionization to adjacent QDs. This process is continued until the charge recombination is directed through two wide bandgap transport layers. Eventually, excitons are formed in pairs of adjacent QDs resulting a radiative emission of narrow wavelength. Further, adding an insulating layer between QD layer and transport layer provided boost to quantum efficiency of QLEDs. These additional layers provided stability to QD charging and carrier imbalance, earlier prevailing due to electron and hole injection inconsistencies [21]. On the other hand, multiple layers as wide bandgap transporter and insulation gradually raised the need of higher electric field to operate and deteriorate the photoelectric property of QDs such as operational stability and emission efficiency (Fig. 4).

2.2 QLED Design

An ideal limitless performing QLED shall possess optimum balance of carrier injection capable of suppressing charge quenching effect and provide an inert interface from oscillating hindrances rising due to charge transport layers. Determining these two fundamental limitations of QLED designing an immediate development to irradicate quenching due to charge injection and charge transport layer materials is demanded. An efficient way to achieve charge balance is by minimizing overpopulation of electrons in transport layers. As per reports, using poly(methylmethacrylate) (PMMA) as an interface to inorganic ZnO/QD layer, the advantage was limiting excessive electron injection by reducing interaction sites between ZnO and QD layer. This optimization resulted in achieving EQE of 20.5% with luminance of 100 cd.m^{-2} for over 100,000 h. Apart from PMMA, other organic insulating layers namely CsCO3, Al2O3, Polyethylenimine (PEI) [22–25], and Polyethylenimine ethoxylated (PEIE) [26–29] have also been reported with significant improvisation in lifetime or luminance.

Charge transport layers have a crucial role in QLEDs and the interface interaction between ETL/HTL with QD layer is usually a retarding behavior in case of inorganic transport layers. To counter these hinderances an Al_2O_3 passivating layer is introduced to NiO layer so that NiOOH passivation could be achieved and quenching of QD emission is minimized. LiF was also introduced in similar understanding to control diminishing emissions, as a result an improvement of 6.52% with EQE of 21,600 cm.m^{-2} was observed [30]. Another critical aspect related to Hole transport layers is hole-induced quenching, this phenomenon is gradual and increases

with lifetime of QLEDs. Inserting 1,3,5-tris(N-phenylbenimidazole-2-yl) benzene commonly known as TPBi as hole separation layer between QD/HTL was considered. This improvisation was closely studied for InP based QLEDs and surprisingly 100% quantum efficiency was achieved [31]. Inorganic separation layers are then being explored to overcome lifetime enhancement of QLEDs that can support unwanted leaching due to heating and recoil effects caused after elongated operations.

Formulating another vital aspect to enhance QLEDs emission is excessive exciton formation at interfacial layers, causing an unwanted charge field. The generation of unbiased fields causes randomization of charge transport mechanism or even pseudo-splitting of device architecture in several fragmented layers. This scenario is fatally alarming that can invoke inevitable failure to the overall device. In order to maintain proper functioning and avoid such circumstances, QD emissive layer shall be introduced away from such p-n junctions having potential to form strong charge fields. This strategy is advantageous in two significant manners i.e., firstly, the photo emission wavelength is unaltered by charge clouds and secondly, the electron path is evenly populated to support emissive properties. There are several other measures to optimize QLEDs performance depending upon their application interests, some of them are detailed in following sections.

2.2.1 III–V QLEDs

The elements from III and V group of periodic table have ample of exciton building combinations, among them InP is one configuration having ~1.35 eV bandgap [32] and excitonic Bohr radius of ~10 nm [33]. Synthesizing InP QD was a reckless task to researchers from several years, until coordination solvents could impart a role. Initially trioctyl-phosphine (C24H51P) was considered a strong coordinating solvent but due to its limitation in synthesis process as long reactivity time and uneven QD size distribution, more suitable methodology came up. Incorporating 'In-carboxylates' with a non-coordinating solvent significantly reduced the reaction time with fairly even size distribution. Further modification in reaction method led to formation of single shell InP/ZnS QD nanostructure with as high 70% QY in PL and ~45 nm in FWHM of green emitters. The efficiency for other emitters could be improvised with similar synthesis protocol but was limited due to finitely thin ZnS outer shell and the lattice mismatch between core and shell layer. To eliminate such hinderance an intermediate layer was proposed which could effectively thicken the overall structure and minimize lattice mismatch. The proposed layers were GaP, ZnSe, and composition-gradients of ZnSeS. These modifications crucially functionalized the PL QY to 85%, 82% for green and red QLEDs respectively.

Another aspect was to monitor losses due to charge transport layers; balancing charge injection and optical quenching is of critical interest. The competing interests to under-incline these losses were handled discreetly, imparting organic solvent and inorganic counterparts were implied. Using ZnO nanostructure as charge carrier in transport layer provided immediate push in emitter efficiency. This was later devised with multiple configurations in inverted QLEDs. Multi-shelled structure

comprising InP/ZnSeS/ZnS QD that increased illumination to a record 70%. On similar notes using ZnMgO as carrier transport layer dopant, that raised the illumination to 10,000 cd/m [2] and current efficiency of 4.4 cd/A. with these many improvements projected in InP QD based LEDs the highest electroluminescence quantum efficiency ranges up to 3.4–4.4%, 2.5–3% for green and red QLEDs respectively. This provides considerable room for further improvisation and adaptations for III–V group based QLEDs.

2.2.2 II–VI QLEDs

It was difficult to get high-quality non-Cd blue QDs with the requisite emission wavelengths of 450–460 nm, which are appropriate for using QLEDs in displays and lighting. The new class of QLEDs are widely acknowledged for deep and ultra-blue emitting light sources. With the innovation of InP QDs, a new understanding was developed for such low bulk bandgap quantum confined nanostructure ranging below 1 nm. Many multi-cored quanta shelled nanostructures have been investigated since then for achieving higher quantum yield, yet the maximum efficiency achieved is 5–40%. Another prominent ZnSe QLED have attained immense attention in past decade with astounding QY. The PL emission ranges from 440 to 455 nm but with limited QY, whereas short wavelength emission within 420–430 nm have been utilized for many high-end applications due to its reliable QY of 83%. Despite reasonably high QY lesser reports is available on ZnSe QLEDs, but several different architectures have been implied among those reports. An inverted architecture using ZnSe QLEDs was reported to have QY of 60% with peak luminescence at 1170 cd/m [2] and current efficiency of 0.51 cd/A.

2.2.3 IV QLEDs

Carbon and Silicon are the top choices in single element based QLEDs owing to their quantum confined zero-dimensional structures. Interestingly, both these elements can emit from visible-to-far infrared region and the optical property is highly dependent on confinement dimensionality or size specific. The very primitive carbon-dot based QLED was reported to have luminance of 35 cd/m [2] and QY higher than 50%. The experimental understandings grew with graphene QDs with remarkable performances, as ETL materials, separation membranes, and emitter dopants as well. Eventually many reports came-up detailing the impact of Boron (B), Oxygen (O), Nitrogen (N), Phosphorous (P), Sulphur (S) doping in Carbon and Silicon QDs nanostructures. Boron is used to increase the LUMO levels, whereas N, P, and S are doped to enhance the HOMO levels. Introducing these dopant elements during synthesis results in developing p-type or n-type electronic vacancies to overall nanostructures. It was evidently supported that doping of N to carbon QDs significantly minimizes the LUMO–HOMO gap, resulting in a considerable red shift in photoluminescence.

Similar photo-emission phenomena under UV source are equally observed with uni-metallic nanosheets as well in earlier reports [34, 35]. Later higher functional groups as amine, phenylenediamine, aminophenazine, and others were explored as ligands to carbon-dot LEDs. Using o-phenylenediamine (O–PD) outperformed many deep red emitting QLEDs with two-photon red fluorescence with emission at 630 nm and 680 nm. In another report protonation of 2,3-diaminophenazine (2,3-DAPN) helped in achieving sharp and narrow red peak of ~20 nm width [36]. Similar reportable emissions were observed with Si-based QLEDs, their red sifted emission had much higher intensity but limited photon lifetime. This part of group IV QLEDs still require many more evidence to underlay fundamental phenomenon between ligands and metallic core that improves multiple excitons within visible electromagnetic spectrum [37].

3 QLED Applications

In recent years QLED has captivated limitless technological options filling the improvement gaps of data communication, quantum computing, displays, sensors, and many other applied research interests.

3.1 Sensors

The devices capable of measuring alteration in standard atmosphere that may occur due to changing temperature, pressure, gas concentration, healthcare scenarios and many other applications require high-end calibrated sustainable methodology promised through using QLEDs. Intriguing demands of high performing sensors in industrial and healthcare scenarios have brought the need to develop non-invasive methods to counter ambient changes caused due to physical factors. Here are several studies reported by various scientific groups that developed different sensors.

3.1.1 Temperature Sensors

These are the newly developed devices based on QLEDs to record the changing degree of heat in an environment. The fundamental working of such temperature sensors is based on temperature dependent photoluminescent property. Initially two different carbon dot based fluorescent nanomaterials were studied for this application, the PL wavelength differs ~20 nm from each other and eventually temperature of incident surface was recorded. The reversible change in fluorescence wavelength of nanosized carbon dots could visually detect the change in relative PL emission that is characteristic dependent to stimuli from temperature of subjected surface [38]. These PL dependent temperature sensing shortly gained immense interest due to highly non-invasive and contact-less method that became need of hour in several industry

and healthcare procedures [39]. Later several studies were made to increase the sensitivity and temperature sensing range up to 300 K. In the newly surfaced scenario, carbon dots grafted using $CaAl_{12}O_{19}:Mn^{4+}$ hybrid phosphor could effortlessly read temperature variance from 80–300 K [40]. To further embark reliability in sensing two fluorescence methodology was studied, where a blue phosphor with yellow-green emissive layer was developed and the latter was extremely temperature sensitive. This technique improvised visual detection method, as the temperature of incident surface rises that induces gradual decrease in yellow-green emission intensity and finally blue color was seen contrasting bright enough.

3.1.2 Gas Sensor

This is another critical aspect of sensing technology worldwide; gaseous analyte monitoring devices require stringent measuring capabilities in ppb scales. This requirement is readily projected by multiple industries at a specific volume. The modeling of device should be carried out in a manner to facilitate analyte molecule or atom to surface at reactivity site of nanomaterial. Gas sensing is fundamentally a chemical reactivity monitored at sub-atomic levels. Firstly, a highly sensitive nanomaterial is synthesized having affinity towards the gaseous content of a specific element. This specificity is later quantified in terms of parts per million/billion (ppm/b) ratio, when present in an ambient environment. In every prerequisite of gas sensors require them to monitor a thermodynamically specific class of gaseous element in an environment composing many mixed or adulterer elemental vapors. Due to incompetency of chemical sensors in real-world scenarios to regulate gaseous composition, QLED based sensors prompted and demonstrated a remarkable efficiency in addition to longer sustainability. Photoluminescent QDs are considered for fabricating high-end gas sensors for heavy-duty applications. The methodology is based on Aggregation induced emission or quenching in most of QLEDs based gas sensors. As the analyte element interacts to nanomaterial surface, it induces certain change in optical properties that are recorded by using QLEDs. These QLEDs are immensely precise in collecting the change of intensity which is calibrated in terms of ppb concentrations for respective gaseous element. Many different sensing platforms are developed for gases derivatives of Oxygen, Phosphorous, Ammonium, Sulfur, Nitrogen, and others depending upon the quantification needs [41–43].

In reports CD/SBA-15, a hybrid nanomaterial synthesized by using mesoporous activity of ethyl orthosilicate in conjunction with carbon dots that possess fluorescent properties [44]. The sensitivity of CD/SBA-15 was concentration dependent that induces an inversely proportional relation with fluorescence intensity. Another interesting report using dual band emission i.e., blue, and red region wavelength were induced for quantifying Oxygen concentration. A hybrid of $CaSiO3:Eu3^{+}$ was synthesized and the overall composition was found to have high affinity towards oxygen [45]. The method reported a minimum response time of 4–7 s and recovery period of 18–29 s. Various other gaseous elements and mixtures such as NO_x,

Methane (CH_4), petroleum gases are also studied and quantified in close to pico-molar range using QLEDs.

3.2 Data Communications

Data communications limitations related to speed and capacity have generally been addressed with semiconductor lasers. For future applications in human-centric systems, short-distance communications, and indoor wireless data services, alternative solutions have been explored due to the comparatively higher production costs and the very complicated driver circuits of lasers, as well as eye safety concerns. A low-cost and high-power substitute are Quantum dot light-emitting diodes. Particularly for Internet of Things (IoT) and 6G technologies, as well as in moderate- to high-speed photonic interconnects, visible light communications (VLC), underwater communications, and precise indoor locating applications, LED-based networks are anticipated to be widely deployed [46–48].

After an electrical signal is received in a QLED communication connection, charge injection, transport, and recombination produce the equivalent optical signal [48, 49]. An LED's usual equivalent circuit may be reduced to a single diode resistor (R_d), capacitor (C), and series resistor (R_s) network. The series resistance is the equivalent resistance of the total resistance linked to the contacts, wire, and drive circuit, which is anticipated to be a constant value, whereas the diode resistance fluctuates nonlinearly with the applied electrical field. The diode capacitance results from charge buildup at the interfaces between several functional layers and the device's dielectric behavior. As a result, the RC effect, charge transit, and recombination are all delayed in time. The mathematical relation between injected current I(t) and applied voltage V(t) is expressed as:

$$\frac{\partial I(t)}{\partial t} + \left(\frac{1}{R_d} + \frac{1}{R_s}\right)\frac{I(t)}{C} = \frac{1}{R_s}\left(\frac{\partial V(t)}{\partial t} + \frac{V(t)}{R_d C}\right)$$

Here, RC time constant be expressed as

$$\tau_{RC} = \frac{C}{\frac{1}{R_d} + \frac{1}{R_s}}$$

Frequency response of a LED is denoted as

$$f_{-3\text{dB}} = \frac{1}{2\pi\tau}$$

where, τ shall be corresponding value of either lifetime carrier τ_C or τ_{RC} and depending on whatever component is the primary limiting factor for modulation

performance. Here, the modulation frequency at which the electrical power available from a detector is cut in half is described as the −3 dB modulation bandwidth $f_{-3\,\mathrm{dB}}$ of an LED. The frequency at which optical power is equal to half of its unmodulated value is known as the corresponding 3 dB optical bandwidth and is denoted by $\sqrt{3(2\pi\tau)^{-1}}$. However, compared to III–V semiconductors, which have substantially higher carrier mobilities (for example, 10^2–10^3 $\mathrm{cm}^2\ \mathrm{V}^{-1}\ \mathrm{s}^{-1}$), fast LEDs based on developing materials, particularly those with organic functional layers, have much lower carrier mobilities. The time-period of injected carriers drift from the terminals to recombination region is charge transit time τ_{tr}—that is found crucial for optimizing high-speed data communicating QLEDs.

3.2.1 Light Fidelity (Li-Fi)

The communication technology expected to surpass Wi-Fi in terms of data transmission rates and users to uni-station will be Light Fidelity (Li-Fi). In general, the use of visible light radiation as data carriers in any given area of interest is known as Li-Fi. The connections to several different users in terms of internet of things (IoT), vehicle guided system, mobile users, and computing units are displayed with relation to Li-Fi. Initially the technique was deployed to a narrow remote purpose such as digital controller units of household appliances like air-conditioners, television controllers, etc., but considering operational power usage; this technology gained interest. Utilizing for mass communication, the head (Tx) access point is positioned at central light field, and other receivers (Rx) are dissipated over a large area of coverage. The transmitted data packs are passed using multiple QLEDs or wavelengths in visible light channel (VLC). Then Infra-red (IR) wavelength is used by receivers to communicate back with access point head in center. The multiple users in Li-Fi communication are marked respectively (Fig. 5).

There are numerous advantages to the QLEDs based data communication technology over conventional wireless data transmission methods such as WI-Fi and radio communication. The InGaN/GaN QLEDs have reportedly crossed benchmark of one gigahertz data transmission bandwidth in past few years. Currently the transmission rates have surpassed 4 Gbps rate using blue emitting Al/Mg doped GaN by *L Wang et. al.,* in year 2021 [50].

3.3 QLED Displays

These days technology has boosted multi-dimensional arena where information must be presented to audiences. The vastness of such information presenting surfaces commonly known as digital displays, ranges from deep sea to far-space applications. These displays require different viewing features due to the operational circumstances. for instance, the deep-sea display requires low luminescence with higher

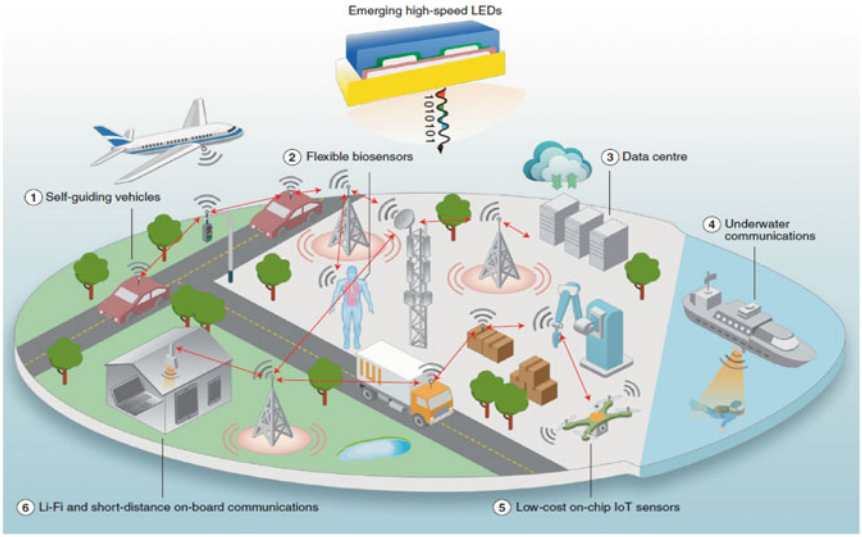

Fig. 5 The communication channel propagation in Light Fidelity (Li-Fi) using QLEDs as source of light. Adapted from [49]

dpi clarity whereas, open sky displays need high luminescence with 8 K resolution. All these prerequisites for concerning display applications are distinctive from one another. In this section, three different types of displays namely Virtual and Augmented reality (VR/AR) displays, Wearable displays, Stand-alone displays are discussed (Fig. 6).

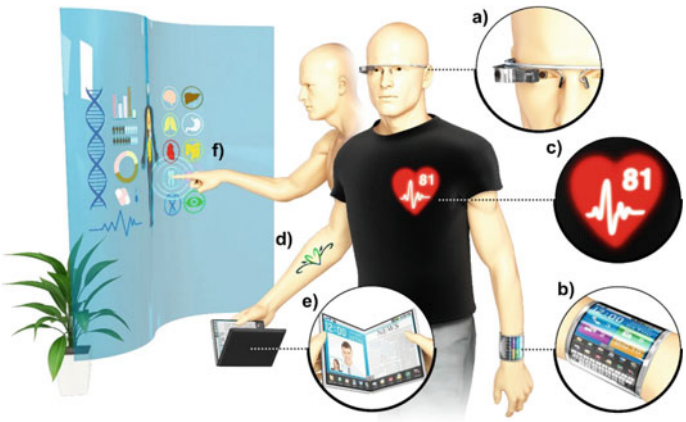

Fig. 6 Future of flexible and wearable displays. **a** Smart glasses with AR/VR. **b** Smart watch with wearable biosensors. **c** fabric display. **d** Ultrathin electronic tattoo. **e** Bendable display. **f** Transparent smart windows. (Adapted from citation [51])

3.3.1 VR Display

These displays are also known as the next generation displays, due to their size and high-definition standards. This class of displays require very high motion picture response time (MPRT) to minimize the motion blur and lowest operational power. These two challenges are met by using QLEDs that can outperform various other display technologies like LCD and TFT. An interesting aspect is VR displays are operated at highly dark and near-vision vicinity of human eye and thus a lower peak luminance of 150–200 cd.m^{-2} is optimum. An added advantage is lower luminance will consume less power but increases error probability of dark spots under high MPRT. This challenge is overcome by moderating chip size between 5–7 μm on 2-inch panel with nearly 3000 ppi (pixel per inch). High luminance is vital for AR displays due to:

a. The information commonly displayed is directly over-portrayed to near environmental backgrounds and thus optimum ACR is required.
b. Space applications requisite high luminance to deliver same luminosity flux the VR display effortlessly delivers same information in lesser luminance with better MPRT.
c. Fast MPRT demands high instant luminance for chip size <7 μm. Organic displays have inversely proportional lifetime to luminance intensity and hence QLEDs have bagged multi-fold advantage to conventional displays.

An example of such lucrative display was reported having ultra-fast MPRT with 5644 ppi and 3500 cd.m^{-2} luminance by *Lu et al.*, in 2019 [52].

3.3.2 Wearable Display

These displays are now commonly used for plenty applications and have gathered ample user interest towards them. The technology backstage is far more versatile than ordinary displays, patternable flexibility is the key to achieve lucrative display characteristics. Flexible electronic devices are fabricated more distinctively than flat or thin electronic devices. Maintaining the robustness of flexible electronic materials requires polymeric assistance that can provide optimum stretchability and retainability. In case of wearable displays:

a. The light source must be arranged as 2D array and thus paving path for emissive displays seen today.
b. The light must possess remarkable off-axis performance, color shifting and ACR coherence are main factors to consider.

The deviation of color rendering in wearable displays was a major challenge in ambient environments that demand high luminance. QLEDs were impregnated with organic ligands to primarily suppress these challenges. Using various InP/ZnSe QD nanomaterials have been reported to dramatically increase the ppi of such wearable displays. Incorporating ITO electrodes over flexible PET surface is an initial step

towards developing flexible display [23]. Keeping a track of flexibility fragile ITO was a limitation due to minimum bending radius was later a research subject for flexible displays. eventually Polyimide based electrode material were developed having an average thickness of 18 nm, that acted as semitransparent top electrode for EL. These developments were later used to develop EL-Tattoo like displays by Choi et al in year 2015 [53]. Use of parylene-C films was later approved by Food and Drug Administration (FDA) as good interface for biocompatible skin displays. Wearable displays also required precise waterproofing to expand the applicability. Hence, high IP rating films were introduced as covering or protective layer over electrode layers. More and more intriguing applications of QLED based wearable displays can be seen in the near future.

3.3.3 Standalone Display

As the name suggests these are the displays observed for regular utilizations from household, vehicle, commercials, and industrial purposes. These displays have been part of regular activities to display numerous forms of information which may include high speed motions. In conventional displays the limitation was operation temperatures that caused degradation of information displayed. The advanced technology using LCDs began to fall apart due to crystal ageing and limited viewing angles. Newer versions were introduced with wide viewing angles and a high dynamic range that could cope with the limitations of displaying information. The next generation displays have ultra-high dynamic range and far better resolution up to 8 K readily available for consumers. Eventually these newer displays have found emerging applications as vehicle cluster panels, space flight panels, aircraft displays, and others which now have stand-alone functioning in fast moving scenarios. These utilities are comparatively new for standalone displays and bought new challenges as turbulence handling, rough environment, shock proofing, and lower operational power. QLEDs have delivered substantial support in all the physical conditions due to robust capabilities and far-low operating potential. QLEDs are further being explored to grow in expectations such as HDR performance, field of view, color corrections, and even transparency for head-up display (HUD).

4 Conclusion

The QLEDs in recent years have gained enormous scientific advancements that have promoted multi-industrial applications all around the globe. Quantum dots-based light emitting diodes are highly versatile and application dependent. The electroluminescence of QDLEDs is classified by three different working methods namely, Energy transfer, charge injection, and field induced. Each of these methods have certain advantages one over the other and thus independent designs. The electron and hole transport layer are the two common interfaces in every device, and their

compositions vary from organic to inorganic constituents. Numerous studies have been performed to enhance the robustness and diverse functioning of QDLEDs. The improvements are related to electron mobilities, charge separation, and QD-sandwiching procedures. Achieving over 80% PL quantum yield has inspired to develop many applied technological devices for several sectors. The devices are used for sensing physical factors such as temperature, pressure, movements, etc. but not limited to these. Eventually gas sensors were developed that are capable of working at elevated temperatures for multiple gases like Oxygen, Nitrogen, Sulphur, etc. Another remarkable achievement of QLEDs is in display and communication technologies. Modern communication methods such as Ligh Fidelity (Li-Fi), internet of things (IoT), and others are dependent on high frequency QLED-based devices. These enhanced technological applications are expected to soon be expanded in space and aviation industry.

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Quantum Dot Composites for Solar Cell Application

Sampurna Mukherjee, Aaishiki Saha, Ankita Chandra, and Sourav Sarkar

Abstract With the ever-increasing energy demand, solar power as an infinite source of renewable energy without any adverse effects to the environment is a plausible alternative to the conventional energy sources. Solar cell is a photovoltaic device, which turn sunlight, the most fascinating renewable energy source, into electricity, has been extensively studied by a sizable number of researchers. In research labs, several types solar cells have been created, and significant progress has also been made towards commercial manufacturing. Most solar cell technologies, except for Si solar cells, are still far from being used in practical applications because of their generally low power conversion efficiency (PCE), lack of long-term stability, and/or harmful compounds used in the components or during processing. The principles of solar cells were covered in this chapter, from early technologies up to the present day. The identification of novel materials has enabled the development of new solar cell technologies with increasing efficiencies. A fundamental description of several solar cell technologies is provided. The crucial special aspects of the nano crystal QD Solar Cell have been large discussed in this chapter. After discussing the quantum heterostructure, the various designs required for the QD solar cell have been briefly explained. The shortcomings and drawbacks of QD Solar Cells have also been focussed on. After that, the top-down and bottom-up synthesis processes for the QD solar cell have been discussed which includes many methods like Reactive-ion etching or wet chemical etching, Focused Ion Beam Technique (FIB), Electron-beam Lithography, Wet-chemical methods, Sol–gel Process, Microemulsion, Hot-solution Decomposition, Microwaves, Hydrothermal Process, Vapour-phase method etc. Among all the discussed techniques, Hot-solution decomposition method is the cost-effective due to high temperature.

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1 Introduction of Quantum Dot Solar Cell and Its Composites

The Silicon Solar Cells were first developed in the year of 1953 for the purpose of developing communication in Satellites and Space Crafts. Solar Cells which are presently used in large-scale as Renewable Energy Sources were first developed in 1973 with a standard technology. Mainly there are three Generations of Solar Cells primarily termed as first, second, third generation solar cells [1, 2].

The single crystal Solar Cell comprises the First-Generation Solar Cell. The First-Generation Solar Cells can perform at high temperatures with a high efficiency of near about 20% and they are highly stable to atmospheric changes. The only drawback of them is the high cost preventing the large-scale use of them [3, 4].

After single crystal solar cells, the thin film technologies have been developed which are classified/termed as the Second-Generation Solar Cell. Amorphous Silicon which is also called Hydrogenated Amorphous Silicon mainly comes under the category of Second-Generation Solar Cells. It has an efficiency of around 10% which is lesser than the First-Generation Crystalline Silicon. The only beneficial quality of the Second-Generation Solar Cell as compared to the preceding generation is its less manufacturing cost which can be used in Commercial Solar Panels for better fabrication [5].

Further improvement in technology leads to the discovery of the next generation of solar cells named as The Third-Generation Solar Cell. This generation of solar cell includes Dye Sensitized Solar Cell Technology, Organic Solar Cell Technology, Quantum Dot Technology etc. The Dye Sensitized Solar Cells are much more cost-efficient in terms of manufacturing and they are comparatively less toxic. This kind of Solar Cells displays a good performance even in light conditions and they possess a good incident angle. In addition, they can be used in low intensity and are very light -weighted, flexible semi-transparent and bifacial material. The main disadvantage is its efficiency which is less than 12% [6].

Next, the evolution Quantum Dot Solar Cells takes place. QDs were first discovered in glass crystals in the year 1980 by Russian Physicist Ekimov. However, Quantum Dot Technology had been advanced after 1984 when the Scientist Luis Brus obtained the size and band gap relation in the shape of a Sphere model of the Semiconductor Nanoparticle with the bulk Semiconductor in the form Wave Function. The speed becomes much faster after the synthesis process of Colloidal CdX (X = S, Se, Te). CdX is the most extensively studied QDs due to the excellent Optical and Electrochemical Properties. QDs are minute semiconductor particles with a size varying between few nanometres. The speciality of QD particles is that they have specific optical and electronic properties which are different from the other particles.

By changing the size of QDs we can tune the shape as well as optical and electrical properties of the material. For their excellent absorbing quality, they play a pivotal role in the third generation PV cells like QDSSCs. The quantum dot cells attained the remarkable efficiency of maximum power conversion of 66% approximately. Moreover, Quantum Dots have other properties as well. Among these the most exceptional property of QDs is that with decrease in size of the nanoparticles, we can notice the energy of lowest quantum confinement levels of electron hole pairs increases with increasing band edge energy for optical transition. In a quantum dot solar cell, quantum dots are typically embedded within the active layer of the cell. This integration allows the quantum dots to absorb a broader range of wavelengths of light, including those beyond the visible spectrum. The tunable bandgap of quantum dots enables customization of their light absorption characteristics, making it possible to match the solar spectrum more effectively. One key advantage of quantum dot solar cells is their potential to mitigate energy loss by facilitating the efficient transport of charge carriers. The quantum dots can be engineered to enhance the separation and collection of electrons and holes generated by the absorption of sunlight, leading to improved overall energy conversion efficiency. Researchers are actively exploring various materials and fabrication techniques to optimize quantum dot solar cells for practical applications. This technology holds promise for advancing the field of solar energy by addressing limitations associated with traditional solar cells and opening new possibilities for more efficient and versatile solar power generation [7–19].

Quantum dot composites in solar cells represent a cutting-edge technology that leverages the unique properties of quantum dots to enhance the efficiency and performance of solar energy harvesting. Quantum dots are nanoscale semiconductor particles that exhibit quantum mechanical properties, including size-dependent tunable bandgaps and high absorption coefficients. In the context of solar cells, quantum dots are integrated into composite materials, often in the form of thin films or coatings, to create what is known as quantum dot composites. These composites can be incorporated into various types of solar cells, such as thin-film solar cells or third-generation solar cells, to improve their light-absorbing capabilities and overall energy conversion efficiency. The key advantages of using quantum dot composites in solar cells include their ability to absorb a broader spectrum of light due to the tunable bandgap, enabling better utilization of sunlight. Quantum dots can be engineered to absorb specific wavelengths of light, allowing for customization to match the solar spectrum. Additionally, their small size facilitates the transport of charge carriers, reducing energy loss and improving the overall efficiency of the solar cell. The development of quantum dot composites in solar cell technology holds promise for addressing the challenges associated with traditional solar cells, such as limited absorption range and efficiency. Researchers and engineers are actively exploring ways to optimize quantum dot composites for commercial applications, aiming to create more efficient and cost-effective solar energy solutions for a sustainable future.

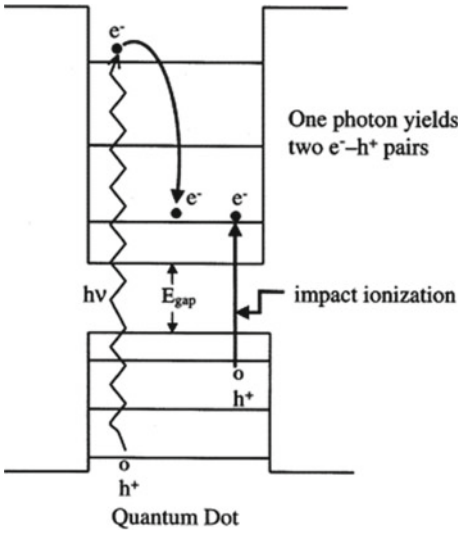

Fig. 1 Impact ionisation (inverse Auger effect)-based increased photovoltaic efficiency in QD solar cells. (Reproduced with permission from Nozik, A.J., 2002. Quantum dot solar cells. Physica E: Low-dimensional Systems and Nanostructures, 14(1–2), pp. 115–120) [20]

2 Quantum Heterostructures

A Quantum heterostructure is defined as the heterostructure in which the size of the nanoparticle has a limitation for the movements of the charger carriers in quantum confinement. It helps to form the separate energy levels in which the carriers can exist. In comparison/contrast to bulk materials, quantum heterostructures do have a better density of states. It has a vital role in the fabrication of small wavelength light emitting diodes, diode lasers and other optoelectronic applications. Quantum Dot Nanoscale Semiconductor Heterostructures are a type of materials that can be well integrated into solar energy conversion devices. This kind of structure can identify and synthesize the given designed materials with their controlled structure and size and show the morphology for different systems individually [21–29] (Fig. 1).

3 Synthesis of Quantum Dots

The two main processes by which we can synthesize Quantum Dots are (i) Top-down approach and (ii) Bottom-up Approach. By applying the former technique bulk materials are gradually reduced to form quantum dots. The latter method mainly includes the wet-chemical and vapor-phase techniques which involve the self-assembly of nanoparticles, aggregation of monomers, wet chemistry, decomposition (Fig. 2).

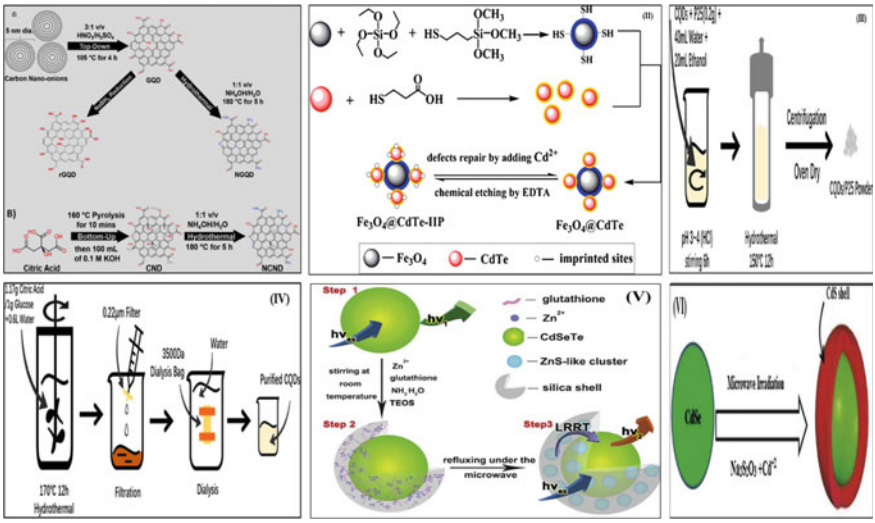

Fig. 2 Different synthesis techniques of quantum dots (Reproduced with the permission from (1) Pillar-Little, T.J., Wanninayake, N., Nease, L., Heidary, D.K., Glazer, E.C. and Kim, D.Y., 2018. Superior photodynamic effect of carbon quantum dots through both type I and type II pathways: Detailed comparison study of top-down-synthesized and bottom-up-synthesized carbon quantum dots. Carbon, 140, pp. 616–623. (2) Luo, X., Guo, B., Wang, L., Deng, F., Qi, R., Luo, S. and Au, C., 2014. Synthesis of magnetic ion-imprinted fluorescent CdTe quantum dots by chemical etching and their visualization application for selective removal of Cd (II) from water. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 462, pp.186–193. (3) Singh, R.K., Kumar, R., Singh, D.P., Savu, R. and Moshkalev, S.A., 2019. Progress in microwave-assisted synthesis of quantum dots (graphene/carbon/semiconducting) for bioapplications: a review. Materials today chemistry, 12, pp.282–314. (4) Shen, T., Wang, Q., Guo, Z., Kuang, J. and Cao, W., 2018. Hydrothermal synthesis of carbon quantum dots using different precursors and their combination with TiO_2 for enhanced photocatalytic activity. Ceramics International, 44(10), pp. 11828–11,834) [21–24]

3.1 Top-Down Approach

The Top-Down Approach helps in producing quantum dots of controllable shapes, sizes with desired packing geometrical capabilities, flexibility, and periodicity for systematic nanostructured design in solar cell applications. A few of the synthesis techniques under this approach have been briefly discussed as follows:

- **Reactive-ion etching or wet chemical etching**

In this process, a radio frequency voltage is applied to create plasma that eventually breaks down the reactive molecules into more reactive fragments inside the etching chamber. After that with the help of masking selective etching is done accordingly. This process is used to produce closed-packed arrays of QD semiconductors [25, 26].

- **Focused Ion Beam Technique (FIB)**

The fabrication of QDs with high lateral precision with dimensions below 100 nm can be achieved by this synthesis technique. They use highly focused beams from molten metals directly on the surface of the semiconductor substrate which determines the size, shape and interparticle distance of the QDs. However, it is an extremely slow, expensive, and low throughput process, i.e., unsuitable for mass production [25, 26].

- **Electron-beam Lithography**

This synthesis technique is followed by etching or lift-off offering highly precise flexibility in designing QD devices for solar cell application. This technique involves an electronic gun from a scanning electron microscope to nanopattern the features on the substrate surface with high levels of accuracy [25, 26].

3.2 Bottom-Up Approach

The bottom-up approach involves the production of most QDs with a high degree of accuracy which exhibit good quantum confinement properties. This quantum confinement effect contributes to the material's unique optical and electronic properties, enhancing the solar cells power conversion efficiency.

A. **Wet-Chemical Methods**

This method involves common precipitation processes with careful control over varying parameters such as concentration and ratios of the species and solvent, temperature, pressure, stabilizers or micelle formation, double layer thickness for proper nucleation sites of the QDs [25, 26].

- **Sol–gel Process**

This process is comprised of three main steps i.e., Hydrolysis, Sol formation (condensation), Gel formation (growth) and is one typical technique that is in use for many years for the formation of QDs. It is a very inexpensive process that can be easily used for scaling up the production but involves broad size distribution subsequently resulting in too many defects [25, 26].

- **Microemulsion**

The microemulsion technique follows synthesizing QDs at room temperature by stirring and rigorous mixing to immiscible liquids (normal emulsion i.e., oil-in-water or reverse emulsion i.e., water-in-oil) until they form emulsions. Utilizing surfactants such as triton-X, aerosol OT (AOT), cetyl trimethyl-ammonium bromide (CTAB), sodium dodecyl sulphate (SDS), and aerosol CTAB, it is possible to disperse

nanometre-sized water droplets in n-alkane solutions. Numerous micelles, or very small droplets, are generated in the continuous oil medium because of the surfactants' termination by hydrophilic and hydrophobic groups on opposing ends. The vigorous stirring of these micelles results in the formation of QDs whose growth depends on their size which is again monitored by the molar ratio of water and surfactant [25, 26].

- **Hot-solution Decomposition**

A high-temperature pyrolysis process of organometallic compounds is involved in the hot solution decomposition process that provides sufficient thermal energy to anneal defects, resulting in well-monodispersed QDs. Compared to other processes, the growth of QDs through this method is relatively slower and the size, shape an overall reaction depend on various parameters such as reaction time, temperature, coordinating agents, purity of the coordinating solvents. Though many QDs can be synthesized by this technique, one of the major drawbacks include the toxic effects of organometallic precursors and excessive cost margin due to the use of high temperatures [25, 26].

- **Microwaves**

By Microwave Synthesis, QDs of the dimension 1–5 nm have been successfully produced. The ultrasonic waves or microwaves pass through the precursor mixture through water helping in the dissociation that results in the formation of QDs [25, 26].

- **Hydrothermal Process**

By controlling the temperature, pressure, reaction, and aging time of the reactants inside a stainless-steel autoclave different sorts of QDs of various shapes, sizes, crystallinity can be synthesized [25, 26].

B. **Vapour-Phase Method**

This method involves the growth of QDs by layers of atom-by-atom process, that subsequently appear on the substrate without any nanopatterning. This self-assembly of nanostructure devoid of nanopatterning mainly involves epitaxial growth which is achieved by techniques such as Atomic Layer Epitaxy (ALE), Molecular Beam Epitaxy (MBE), Physical vapor Deposition (PVD), Chemical vapor Deposition (CVD) [B1, B2] (Table 1).

4 Designs for QD Solar Cell

4.1 Optical Design for QD Solar Cell

The quantum confinement effect is the vital concept for QDs. The exciton is confined in zero-dimensional space when the nanoparticles are smaller than the radius of Bohr exciton. Hence the band gap gets increased by decreasing the particle size which in

Table 1 Synthesis of various QDs using different synthesis methods

Synthesis process	Examples	References number
Reactive-ion etching	> The enhanced efficiency of the N-I-P structure amorphous silicon solar cell with nanopillar arrays and CdS QDs layers. The short circuit current is increased from 20.3 ± 0.6 mA/cm^2 to 37.6 ± 0.8 mA/cm^2. The power conversion efficiency is increased from 6.63% to 18.45%. These excellent results are attributed to (i) the antireflection effect of the nano-patterned substrates and the newly rough surface formed by CdS QDs; (ii) CdS QDs absorption and photon down-conversion. The a-Si NPA solar cell with CdS QDs layer is suitable for Si-based solar cells, and other related applications in the future	> [27]
Sol–Gel process	>The sol–gel process approach to spin-coat a thin Si-QDs layer confined in a silica matrix to form composite films. The absorption edge of these films is governed by quantum confinement, meaning that it is a function of the diameter of Si-QDs. The bandgap of these layers of around 1.8 eV is consistent with the use of these films in a tandem photovoltaic cell. Moreover, the bandgap is invariant with Si-QD density in the matrix (in the range 2.0–2.6 10^{18} particles cm^{-3}). The band gap of the nanocomposite layers is not modified by high-temperature treatment for dopant diffusion, which is an essential element for the intended application of solar cells >One step high-temperature synthesis of TiO_2/CdS nanocomposite. The effective heterointerface and high crystallinity, according to scientists, are responsible for the improved visible-light-driven photocatalytic effectiveness. Varying sizes of CdS QDs can control the absorption of TiO_2/CdS nanocomposite. This type of quantum-dot-sensitized solar cell, results in an ideal band gap and conduction band potential	> [28, 29] > [29–31]
Microemulsion	>CdS:Mn/ZnS, Mn doped CdS core, epitaxial ZnS shell by reverse micelle process; Particle Size: 1.5–2.3 nm	> [25, 32–37]
Hot-solution Decomposition	>1996: CdSe/ZnS; single-step synthesis; Core: 350 ºC at 1 atm, Ar; Reaction Growth: 310 ºC; Shell: 300 ºC; Particle size: 2.7–4 nm >1997: CdSe/CdS; two-step process; Core: 300 ºC; Shell:100 ºC; Particle size: 2.5–4 nm >1996–1999: InAs/InP, InAs/CdSe; two-step process; Core: 200 ºC; Shell: dropwise addition at 260 ºC; Particle size: 2.5 − 6 nm (InAs); Core/shell: 1.7 nm >2001–2003: CdSe/ZnS, two-step process: Core: reaction and growth: 270º–310º; Shell: slow addition of Zn & S precursor at 180–220 ºC	> [25, 38] > [25, 39] > [25, 40–42] > [25, 43, 44]

(continued)

Table 1 (continued)

Synthesis process	Examples	References number
Microwaves	> During synthesis, CdSe seeds were first formed by the reaction of NaHSe and Cd^{2+}, and then alloyed quantum dots (CdSe–CdS) were rapidly produced by releasing sulfide ions from 3-mercaptopropionic acid as sulfide source with microwave irradiation. The alloyed quantum dots synthesized had good optical properties, the quantum yield was up to 25%, and the full width at half maximum of the emission spectrum peak was about 28 nm >Nanocomposites of graphene oxide (GO) or reduced graphene oxide (rGO) with $CuInGaSe_2$ quantum dots (QDs) synthesized by a microwave-assisted method; bulk CIGSe has been successfully applied in thin film solar cells with ca. 20% of efficiency; synthesis of nano-sized CIGSe and other quaternary quantum dots (QDs), aiming at further improving the efficiency of the solar cells and the reduction of costs by using lower amounts of materials; the nanocrystals of 10 nm average size possessed a large absorption tailing and emitted light at 650 nm >Water-dispersed CdSe/CdS/ZnS core/shell/shell QDs with high photoluminescence and a large Stokes shift were synthesized by a facile microwave-assisted route with the highest quantum yield of 25.4%; fabricated CdSe/CdS/ZnS QDs/SiO_2 composite films were applied to Si solar cells as luminescent down-shifting layers and enhances QDs on the photoelectric conversion efficiency of the Si solar cell by spectral down-shifting as compared to the bare glass substrate, and the maximum achieves 16.14% >The B-GQDs are found to have an average lateral width of B6 ± 2 nm and thickness of B0.2–0.3 nm. Nano ZnO is incorporated into these B-GQDs to prepare a B-GQDs-ZnO composite based high-performance photo-anode for Dye-Sensitized Solar Cells (DSSCs). The results showed the highest power conversion efficiency of B3.7% with these new composite-based DSSCs, showing the potential of this composite in real-cell applications	> [25, 45] > [46] > [47] > [48]
Hydrothermal process	>Zero-dimensional graphene quantum dots (GQD) anchored on reduced graphene oxide (rGO) sheets were synthesized by hydrothermal method. CuS and GQD anchored rGO sheet (GRGS/CuS) composite was prepared by simple spin coating and thermal sulfidation techniques and used as counter electrode (CE) material for CdS quantum dot sensitized solar cell (QDSSC). This synergistically helped to reduce the charge transfer resistance of the composite counter electrode with increased current density and to enhance the photovoltaic performance of bare CuS and GRGS CEs. This synergistically helped to reduce the charge transfer resistance of the composite counter electrode with increased current density and to enhance the photovoltaic performance of bare CuS and GRGS CEs, which has a brilliant future for high efficiency solar cells >One-step approach for the synthesis of carbon dots (CDs); CDs can be conveniently utilized by coating the hybrid solution on proper substrates, etc.; based on the luminescent down-shifting behaviour of the composite with CDs, the power conversion efficiency of polymer-fullerene-based bulk heterojunction (BHJ) solar cell is increased by about 12% due to effective light conversion of near ultraviolet and blue-violet portions of sunlight	> [49] > [50]

(continued)

Table 1 (continued)

Synthesis process	Examples	References number
Atomic layer epitaxy, Molecular Beam Epitaxy, physical vapour deposition, chemical vapor deposition	>Kim et al. synthesized ZnSe/ZnS by Stranski-Krastonov mode using CVD technique in ALE mode of operations (mean dot height 1.9 nm) >For indium arsenic/gallium arsenic (InAs/GaAs) systems, In and as atoms are deposited on the GaAs substrate to form an epitaxial layer. A large band gap material, such as InGaAs, is then deposited on top of the InAs epitaxial layer	> [25, 51–60] > [59, 60]

turn affects the band structure and absorption coefficient of the materials. The light path is an important part for optical design in QD Solar Cell, as light gets reflected at the interfaces by reducing the injected reflection of light and increasing the inner reflection of light. The high refractive index is important parameter for controlling the energy level providing Fresnel reflection at a high order at the interfaces, which is the major reason for light loss in QD Solar Cell [35–63].

4.2 Electrical Design for QD Solar Cell

The fabrication of superior performance in devices is mainly dependent on the electrical properties of QDs as the ratio of the surface area to volume, interdot distance and transportation of the carrier in QD depends on surface modification and short ligand treatments. The ligands also affect the energy states and fermi level carriers are generated by photon energy via doping. The surface modification helps to trap density and the alignment of the energy in the device. The surrounding ligands provide the QD shifting by producing Dipole Moment. Therefore, we can say surface chemistry modification is the main approach for enhancing the efficiency of solar cells [61].

4.3 Stability Issue and Design for QD Solar Cell

The design for the stability is the main role for manufacturing QD Solar Cells. The device of the QD Solar Cell is mainly dependent on the QD materials which comprises of large surface area and organic–inorganic hybrid ligands. QDs synthesized at high temperature possess high crystallinity tend to degrade faster which affects the size dependent characteristics [61, 64, 65].

It can be overserved or noticed the QD solar cell is less efficient than solar cells made up of materials like organics, perovskites and others which are mainly made up of thin films and Si wafers. Hence QDs are insufficient for conversion efficiency so better designs are essential for getting a good result (Fig. 3).

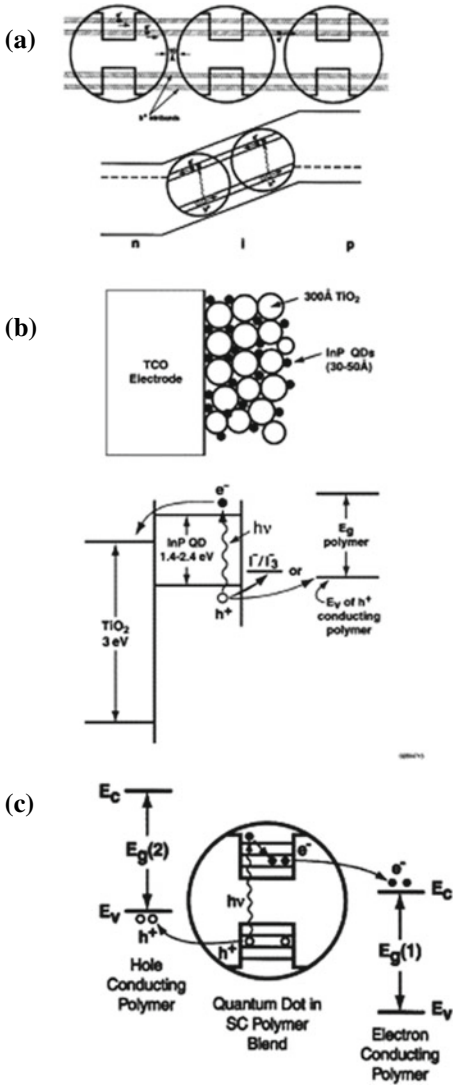

Fig. 3 Different configurations in QD solar cells. **a** a QD array that functions as a photoelectrode for a photoelectrochemical or as the i-region of a p-i-n photovoltaic cell; **b** QDs were used to make a nanocrystalline TiO_2 semiconductor with a wide band gap more sensitive to visible light. This arrangement is comparable to the dye-sensitized solar cell, except that QDs are used in place of the dye; **c** QDs dispersed in a mixture of polymers that conduct electrons and holes. In configurations (**a**), (**b**) and (**c**), Higher photocurrents and better conversion efficiency might result from impact ionisation. In **a**, A higher photo potential could be attained through impact ionisation or hot carrier transport through the QD array's minibands to increase efficiency (Reproduced with the permission from Nozik, A.J., 2002. Quantum dot solar cells. Physica E: Low-dimensional Systems and Nanostructures, 14(1–2), pp. 115–120) [20]

5 Conclusion

In this chapter, advancement in the QD solar cell technology have been briefly discussed. QD Solar cells have been mostly focussed on by contemporary researchers as the fine tuning of the size of quantum dot can lead to change in optical and electronic property. By utilizing this feature of QDs the efficiency of the solar cells can be optimized and in addition other properties of QD solar cells can be modified according to our requirement. The QD solar cells have reached a maximum efficiency of 66% approximately which higher than the solar cells currently used for practical applications. The various synthesis procedures from bottom-up to top-down approach have been focussed on. The size and the morphology of the QDs achieved in each synthesis process have briefly discussed along with the cost efficiency, feasibility, benefits, and disadvantages of each technique. The morphological structure and the size of these dot have a huge impact on the amount of solar light absorption which highly influences the efficacy. However, the QD solar cells are yet to be commercially used due to various shortcomings such as low power consumption, constant power supply, long-term stability which need to be further studied to improve the efficiency of these solar cells and put to practical use.

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Quantum Dots Composites in Supercapacitor Applications

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Abstract This chapter focuses into how quantum dots have been cleverly incorporated into supercapacitor technology. Quantum dots are studied for their potential to improve supercapacitor performance because of their unusual qualities, such as their electrical behaviour that changes with size and the impact of quantum confinement. Quantum dot composites are discussed in this chapter, including their synthesis, characterisation, and electrochemical characteristics. It also explores their potential use in energy storage devices with an eye towards increasing the devices' energy density, charge–discharge dynamics, and durability. This chapter is a great introduction to the intriguing new subject of quantum dot-based supercapacitors, which has great promise for cutting-edge energy storage technologies.

1 Introduction

As traditional fossil fuel reserves appear to be dwindling, concerns have been raised about the future of energy generation and distribution. This unfortunate upheaval has prompted people in recent decades to investigate renewable energy sources like wind, solar, biofuels, etc., which have shown great promise in reducing reliance on traditional fuels like coal and oil. Another possible reason to lean towards nontoxic

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or very lesser hazardous renewable energy sources is the scarcity of coal and oil-like natural supplies and the damaging influence they have on the environment. Experiments have shown that they pose minimal to no danger to the environment; therefore their widespread use is being examined as a means of making daily energy consumption more exact and secure and of putting an end to the unchecked growth of global warming. In order to make better use of various energy sources, the construction of large energy storage facilities became a possibility. There has been a lot of thought put into this, including research into electrochemical energy storage technologies like batteries, supercapacitors (SCs), and fuel cells, all of which are having a profoundly positive effect on the technological landscape and on people's daily lives. Several studies have proposed widespread use of SCs in energy storage applications as a result of their high power density and high device performance. However, batteries have recently gained a better reputation than SCs in large-scale industrial applications due to their dependable energy density performance. However, batteries have a greater production cost than SCs, and SCs can be made even cheaper by selecting the most cost-effective electrodes. Whereas SCs have a very long cycle life, even batteries whose performance has adequate cycles are still not enough to meet the need of advanced electronics. Batteries also have significant limitations in terms of safety, adaptability, and weight. SCs that are both tiny and flexible, and that use a solid-state, semi-solid, or gel-polymer electrolyte, can readily solve all of these problems [1]. EDLC SCs, pseudo-SCs, faradaic-SCs, and hybrid SCs are just some of the common subtypes of SCs. Carbon-based substances are typically classified as EDLCs [2]. Pseudocapacitors are found wherever metal oxides or chalcogenides behave in this way. Furthermore, the metal oxides/chalcogenides used in batteries store their charges via faradaic processes [3].

In the near future, electrochemical capacitors (also known as supercapacitors) will be the most common type of energy storage device due to their high power density, quick charge–discharge rate, long life, and stability [4]. The poor energy density of electrochemical capacitors, especially electrical double layer capacitors (EDLC), presents a significant barrier to their widespread use. Pseudocapacitive materials, such as metal oxide (RuO_2, MnO_2, Fe_2O_3, V_2O_5, Co_3O_4, NiO_x, etc.) nanoparticles (NPs), can be used to boost energy density [5]. Manganese oxide-based materials have attracted a lot of interest because of their abundance, low cost, and nontoxicity among these metal oxide-based compounds [6]. Dispersing graphene in an aqueous solution remains a difficulty for graphene-MnO_2 nanocomposite production, despite the fact that graphene's scaffolding nanostructures and strong electrical conductivity enable large mass loading of MnO_2 and increase the energy density of supercapacitors [7]. Consequently, graphene oxide (GO) has been used in the synthesis of composites containing MnO_2 NPs, as reported by a number of research [8]. However, GO nanosheets' weak electrical conductivity restricts their capacitance as a nanostructured scaffold. To increase the electric conductivity of nanocomposites for supercapacitor applications, GO must be treated with reductants or exposed to light to create reduced graphene oxide (RGO), which can then be used to form electronic conduction channels [9]. However, the production of MnO_2 NPs and their subsequent deposition on RGO can damage the sp2-hybridized carbon structures

of the RGO. Consequently, the use of appropriate reducing agents and the intricate modification of RGO for protection by surfactants or polymers are crucial steps in the composite synthesis process.

Quantum dots (QDs) have gained popularity because of their ultrastable photoluminescence capabilities, tiny size (2–5 nm), and ability to host a variety of functional groups (e.g., hydroxyl, carboxylate, carbonyl) on their surfaces [10–12]. Delocalized electrons are primarily found in the C = C bonds that make up the carbon core of a QD [13–15]. Applications for composites based on quantum dots can be found in many domains, such as biomedical engineering, environmental remediation, photonics, optoelectronics, and electronics [10, 13, 16–23]. Graphene quantum dots and other forms of CDs have recently found use in a variety of energy storage and generation devices, including fuel cells, metal-oxide-based supercapacitors, and lithium-ion and sodium-ion batteries. During the charge–discharge processes in supercapacitors, QD networks in hybrid nanomaterials improve the accessibility of the charged ions, which aids in charge transfer and ionic motion. Supercapacitors and fuel cells can both benefit from the superior capacitance properties and catalytic properties of hybrid nanomaterials synthesised from nitrogen- and sulfur-codoped graphene quantum dots and reduced GO [24].

2 Electrochemical Supercapacitors Based on Carbon Dots

2.1 QDs in Electric Double-Layer Capacitors (EDLCs)

Electric double-layer capacitors (EDLCs), more commonly known as "supercapacitors," are renowned for their high energy density and short charging times. They've found uses everywhere from consumer electronics to alternative energy. Quantum dots (QDs) are one such novel material that has been included into EDLCs in an effort to improve their performance and energy density. Higher energy densities, faster charge–discharge rates, and enhanced overall performance are just some of the benefits that this new field promises. Electric double layers (EDLs) are the fundamental building block of EDLCs, where they develop at the electrode–electrolyte interface. Due to their high surface area and porosity, which give adequate space for ions to accumulate, activated carbon materials have traditionally been utilised as electrodes in EDLCs. Since these materials have a low energy density, scientists have been looking for alternatives, and QDs fit the bill. The unique quantum confinement effects of quantum dots are the result of their nanometer-sized semiconductor particle composition. By carefully manipulating their size and composition, their electrical and optical properties can be finely adjusted. Quantum dots (QDs) added to EDLC electrodes have various benefits. Their increased surface area for ion adsorption is due, in part, to their diminutive size when compared to more conventional materials. This means that the EDLC can store more charges, increasing its energy density. The high electrical conductivity of QDs is also important for the

rapid transfer of charges within the electrode. QD-based EDLCs are ideal for high-power applications like electric vehicles and renewable energy systems due to their fast electron-shuttle speed. QD-based EDLCs are an attractive answer to the energy storage problems of the present because of their high charge storage capacity and fast charge–discharge speeds. QDs in EDLCs also stand out due to their electrochemical stability. QDs demonstrate extraordinary endurance, guaranteeing a lengthy cycle life for the supercapacitor, in contrast to some other high-capacity materials which suffer from degradation over time. Having a battery that can withstand repeated charging and discharging for a lengthy period of time is essential for any application. Significant progress has been made in recent years in the production and integration of QDs into EDLC electrodes. Electrode materials based on QDs can have their characteristics modified in a number of ways, including by solution-based deposition, chemical vapour deposition, and electrodeposition. To further improve the performance of EDLCs, researchers have investigated hybrid architectures, which combine QDs with other materials like graphene or carbon nanotubes. Despite these promising developments, EDLCs still face obstacles in fully utilising QDs. For instance, researchers are working to improve QD integration into useful devices and guarantee their stability through extensive electrochemical cycling. In addition, ensuring cost-effectiveness as manufacture of QD-based EDLCs is scaled up for commercial applications are essential.

Sinha et al. reported the development of a hybrid supercapacitor that is sensitive to light. By fusing carbon dots (CDs) to the surface of SWCNT/ZnO nanocomposites, a hybrid electrode material for the supercapacitor was synthesised. By using the supercapacitor while it is lighted (with UV light), researchers have been able to investigate the optical characteristics of CDs and ZnO [25]. At a scan rate of 50 mV/s, the UV light was found to increase the areal capacitance of the manufactured supercapacitor by 41.38%. It has also been investigated how the supercapacitor system operates. Electric double layer capacitance (EDLC) and pseudocapacitance are both shown to contribute to the total capacitance of the hybrid electrode. Dunn method verification of EDLC and pseudocapacitance contributions yields values of 69.35 and 30.65% for EDLC and pseudocapacitance, respectively. As an electrode material for organic electric double-layer capacitors (EDLCs), commercial activated carbon (YP50f) was doped consecutively to increase its electrochemical characteristics [26]. The sequential doping technique involves applying nitrogen doping following an oxygen doping step. We used the sequential doping technique to create a carbon material with a high electrical conductivity and a large specific surface area. Oxygen doping significantly increased the carbon material's specific surface area, whereas nitrogen doping improved its electrical conductivity. Through in situ polymerization of GO/CDs with pyrrole under mild conditions, a ternary composite comprising graphene oxide, carbon dots, and polypyrrole (GO/CDs/PPy) has been synthesised for usage as electrode active material in supercapacitors. By facilitating electron transport in the ternary composite between the GO film and PPy layer, CDs can lower the internal resistance and charge transfer resistance of the electrode [27]. In addition, the dielectric constant of the ternary composite can be improved by using CDs with a high specific surface area at the interface of GO, CDs, and

PPy. For example, at a current density of 0.5 A/g, GO/CDs/PPy displays a specific capacitance of 576 F/g, and a supercapacitor produced with two symmetric GO/CDs/PPy electrodes possesses a high energy density of 30.1 Wh kg1 at a power density of 250 W/kg. Utilising a straightforward interface self-assembly and subsequent activation technique, 2D porous carbon nanosheet (PCN) with high carbon yield was synthesised from graphene quantum dots present in industrial sulfonated pitch waste. The synergistic impact of electrons and ions is fully exploited thanks to its ultrathin thickness of 20 nm, sufficient specific surface area (SSA) of 2429 m2 g1, high packing density of 0.562 g cm3, and great mechanical flexibility. The structural benefits of this PCN make it suitable for usage as a high-performance EDLC electrode. The high volumetric capacitance of 90.1 F/cm at 0.5 A g1 in pure EMIMBF4 electrolyte, along with the exceptional rate capability C20/0.05 of 71% and the good high-voltage endurance lifetime of 88% after 5000 cycles at 1 A/g, make this material very promising. The highest achievable volumetric energy density is 39 Wh/L, which is quite satisfactory. Furthermore, this approach can offer a fresh viewpoint on the design of other 2D carbon materials. To predict thermal electrokinetics in electric double layer capacitors (EDLCs), Ji et al. suggested a new variational, thermodynamically consistent model. Non-isothermal ion transport with temperature inhomogeneity is modelled using modified Nernst-Planck equations, which are based on the least action principle and maximum dissipation principle of non-equilibrium thermodynamics. An equation for the development of temperatures, including contributions from thermal pressure and electrostatic interactions, is derived using the laws of thermodynamics. The ability of the devised model to capture reversible and irreversible heat production is demonstrated by the accurate prediction of temperature oscillation in the charging-discharging processes of EDLCs in numerical simulations. Simulations using cyclic voltammetry allow for a methodical examination of how ionic size and scan rate of surface potential affect ion transport, heat generation, and charge current [28]. The thermal electrokinetics in EDLCs are seen to have delayed dynamics as depicted by hysteresis diagrams, meaning they cannot keep up with the surface potential at high scan rates. As a result, the physics-based prediction of thermal electrokinetics in EDLCs can benefit from the work we've done. Two commercially available activated carbon electrodes (RP20 and MSP20) for an electric double-layer capacitor have their rate capability predicted using electrochemical impedance analysis. To do this, an equivalent circuit is applied to the ac impedance data, which includes the ohmic resistance and pore impedance of the intra-particle. Fitted impedance parameters are used to profile ionic accessibility into intra-particle pores, and these profiles are then converted into usable capacitance graphs as a function of charge–discharge rate, allowing for characterization of the latter. The impedance analysis prediction of the rate capability matches up well with the charge–discharge rate test result [29]. Composite materials were created by Harper et al. by coating CDs homogeneously over lignin-based activated carbons [30]. After applying N-doped CDs and mixing them with lignin-based ACs at an optimised ratio, the CDs were evenly dispersed across the surface of the extremely porous AC matrix, creating a 3D carbon composite with a CD-decorated interior.

Hydrophilic binding sites produced by CDs on the stable AC matrix give many electroactive sites with outstanding cyclability, rather than optimising the inner porous structure. When it comes to attracting ionic species and solvated electrolyte ions in aqueous environments, the superhydrophilic CD sites in the new design of carbon composites have an edge over the undecorated deep and branched micropores. More aqueous electrolytes can move and diffuse into the porous network when CDs are uniformly adhered to the surface of ACs. As a result, the CDs drastically reduce the length of the ionic diffusion path, resulting in more rapid ionic diffusion and less resistance to mass transfer (Fig. 1). This study suggests a straightforward layout for achieving high-performance SCs with a straightforward production technique. We demonstrated the method's efficacy and the 3D carbon composite's viability through the verification of defining measurements; the latter could be used in a wide range of energy-storage devices, from carbon electrodes for EDLCs and ion-storage batteries to catalyst electrodes for fuel cell and photovoltaic cell applications. CD@AC-12 has a specific capacitance of 191.8 F/g, and it grows by 52% when only half as many CDs are added, supporting the idea that the improved capacitance is proportional to the amount of CDs added. These results suggest that modifying ACs with carbon dots is a promising approach to enhancing their performance, though the corresponding study is still in its infancy.

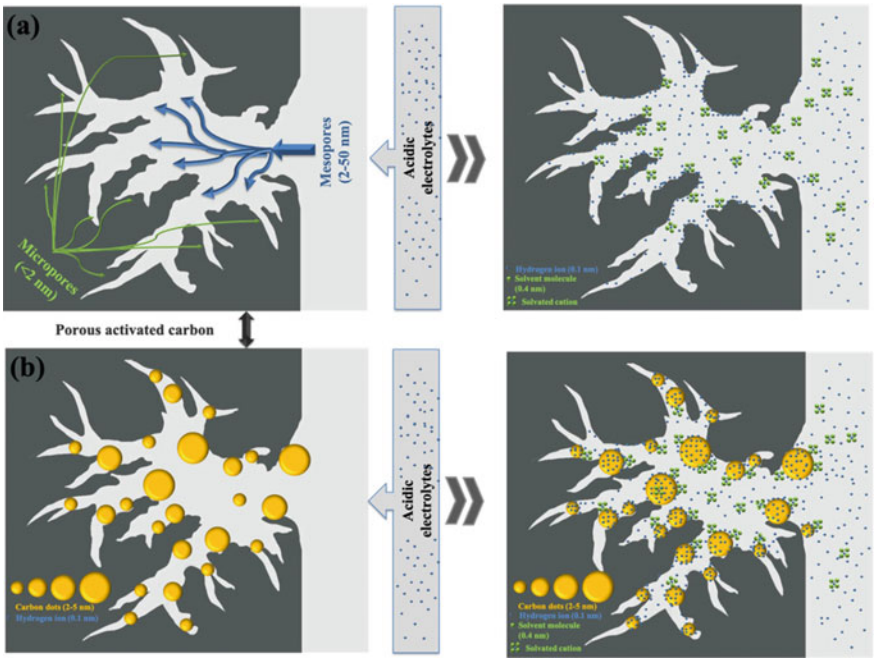

Fig. 1 Schematic illustrations of the charging process in (a) AC and (b) CD@AC-11 electrodes. Reproduced with the permission from Ref. [30] © 2021 American Chemical Society

The EDLC capacitance of GO/CDs/PPy was found to be enhanced [31]. The inclusion of CDs improved the supercapacitive characteristics, as reported by Lima et al. [32].

2.2 QDs in Asymmetric Capacitors (AC)

Asymmetric capacitors, also known as supercapacitors and ultracapacitors, are a major technological leap forward in the field of energy storage. Asymmetric capacitors have a different electrode arrangement than ordinary symmetric capacitors, which store energy electrostatically by amassing similar charges on two conducting plates. Typically, one electrode in such a device will have a large surface area, while the other will have a much smaller one. The primary benefit of asymmetric capacitors is that they are able to store more energy than symmetric capacitors. High capacitance and energy density are provided by the low-surface-area electrode, which is typically formed of a metal oxide, while a considerable amount of charge can be accumulated at the high-surface-area electrode, which is typically made of activated carbon or graphene. Because of this imbalance, we may store energy in a way that is more compact and efficient than either conventional capacitors or batteries. Asymmetric capacitors are well-suited for uses like regenerative braking in electric vehicles and peak power demand levelling in renewable energy systems because of their high charge and discharge rates. Asymmetric capacitors are exceptionally long-lasting and economical because they can withstand thousands, if not millions, of charge–discharge cycles before failing. Many industries, from consumer electronics to aerospace, have a need for high-performance energy storage technologies, which might be met by these materials. Asymmetric capacitors have the potential to revolutionise energy storage, ushering in a more eco-friendly and cost-effective era of innovation.

Micro-supercapacitors (MSCs) are a novel type of energy storage device that has garnered a lot of interest due to their many advantages. However, MSCs still fall short due to their limited operating voltage, slow frequency response, and low energy density. As a result, it is still a significant challenge to devise a method to effectively enhance the electrochemical properties of them by innovating upon the design in a number of different ways. For the purpose of fine-tuning the physicochemical properties of 2D materials, surface and structural engineering techniques such as miniaturisation to the quantum dot scale, doping heteroatoms, increasing the number of structural defects, and introducing rich functional groups are used [33]. Both the molybdenum disulfide quantum dots (MoS2-QDs) and nitrogen-doped graphene quantum dots (N-GQDs) produced in this way make for excellent negative and positive electrode materials. The obtained N-GQDs//MoS2-QDs asymmetric MSCs device demonstrates important characteristics, including a high energy density of 0.55 mWh cm3, long-term cycling stability, a high energy density of 1.5 V (far exceeding that of most reported MSCs), an ultrafast frequency response (with a short time constant of 0.087 ms), and ultrafast frequency response (with a short

time constant of 0.087 ms). There is a lot of room for improvement in the use of binary metal oxides as electrodes in supercapacitors. $NiCo_2O_4$ nanostructures with a three-dimensional flower shape have been obtained through a cheap and straightforward hydrothermal synthesis and subsequent annealing at various temperatures [34]. Nanostructure structure and electrochemical behaviour as a function of calcination temperature have also been studied. $NiCo_2O_4$ quantum dots (NCO-QDs) with a specific capacitance of 362 F/g at a current density of 0.5 A/g were formed in a highly porous, hollow, and continuous structure at a calcination temperature of 300 °C. These nanostructures may exhibit favourable electrochemical behaviour due to their novel, extremely porous, integrated nanostructures, which serve as an excellent electroactive surface for faradaic redox processes. It has also been demonstrated that an asymmetric-supercapacitor made of NCO-QDs as the positive electrode and reduced graphene oxide (rGO) as the negative electrode can store a significant amount of charge (81 F/g at the scan rate of 5 mV/s) and maintain nearly all of that charge after a thousand cycles. After one thousand charge–discharge cycles, the supercapacitor's energy density and power density were measured at a potential window of 2.5 V. The device's power density was 2.22 kW/ kg at a current density of 1.5 A/g, and its energy density was 69.5 Wh/kg. Ni_3S_4 quantum dots (QDs) show enormous potential for supercapacitors because of their unique quantum effects, high specific surface area, high water solubility, and superior stability; nevertheless, the current manufacturing technique is time-consuming, hazardous, and laborious. With the help of polyvinylpyrrolidone (PVP) and ethanol, Zheng et al. reported for the first time a simple and eco-friendly synthesis of Ni_3S_4 QDs [35]. XRD analysis of the impact of the synthesis solvent indicated the synergistic process. The specific capacitance at 1 A/g for QDs showed that they were an appropriate electrode material for supercapacitors. Furthermore, a Ni_3S_4 QD//AC asymmetric supercapacitor (ASC) was built, which provided a maximum energy density of 60.4 W hkg. This study introduces novel strategies for preparing QDs and suggests novel approaches to synthesising nickel sulphide. Suzuki et. al. developed tunnel diodes with a few self-assembled InAs quantum dots encased in symmetric and asymmetric AlGaAs barriers to investigate tunnelling current through individual self-assembled quantum dots. Current peaks due to resonant tunnelling through zero-dimensional states in the individual quantum dots have been seen in the current–voltage characteristics of the symmetric barrier diode for both voltage polarities [36]. Doong et al. produced a V_2O_5 nanosheet (VNS) adorned with 0D graphene quantum dots (GQDs), demonstrating long interlayer distances, high stability, and rapid electrochemical kinetics [37]. Flexible, ultrathin, and lightweight next-generation power sources could benefit greatly from the use of free-standing paper-like thin-film electrodes. Paper-like electrodes made from a silver quantum dot (2–5 nm) modified transition metal oxide (including MoO_3 and MnO_2) are created in this work for use in energy storage. Significant enhancements in electrical conductivity, efficient ionic diffusion, and areal capacitances are observed in the hybrid nanostructure electrodes due to the ohmic contact at the interfaces between the silver quantum dots and the MoO_3 nanobelts (or MnO_2 nanowires). The constructed asymmetric supercapacitors with Ag quantum dots/MoO_3 "paper" as anode and Ag quantum dots/MnO_2 "paper" as cathode and neutral

Na_2SO_4/polyvinyl alcohol hydrogel as electrolyte present significantly enhanced energy and power densities in comparison to those of the supercapacitors without modification of Ag quantum dots on electrodes; present excellent cycling stability at different current densities and good flexibility under cyclic loading conditions [38]. Graphene/carbon nanotube (G/CNT) composites doped with Ni_3S_4 quantum dots (QDs) were synthesised in a single step. Graphene is created by a combination of carbon nanotubes and a Ni catalyst. In addition to preventing Ni_3S_4 QDs from clumping together, G/CNTs can also eliminate the insulating disadvantage brought on by the QDs' interface. Adding G/CNTs to Ni_3S_4 QDs increased the electron interaction, as shown by XPS measurement, and hence facilitated electron transport. In electrochemical tests, G/CNTs were found to boost the rate capability of Ni3S4 QDs from 22 to 82%, and to enhance the specific capacitance of the electrode material by a factor of 4.5 [39]. For use in a high-performance asymmetric supercapacitor device, a three-dimensional (3-D) porous structure based on carbon dot (CD)-supported copper sulphide (CuS)-decorated graphene oxide (GO) hydrogel was developed to increase the specific capacitance, cyclic stability, and energy density of CuS and GO based supercapacitors. Hydrothermal reaction at 180 °C was used to prepare CuS@CD decorated GO hydrogels (CuS@CD-GOH), which were then optimised using a variety of spectroscopic, diffraction, microscopic, and electrochemical techniques. In the 3-D hydrogel structure, CD served as a stabiliser for the CuS nanoparticles and aided in the strong binding of CuS nanoparticles with GO [40]. At a current density of 1 A/g, the CuS@CD-GOH demonstrated a high specific capacitance of 920 F/g. Fabricating an asymmetric supercapacitor with the optimal CuS@CD-GOH as the positive electrode and reduced GO as the negative electrode resulted in the highest energy density (up to 28 W h/kg), longest cycling life (up to 5000 cycles), and highest specific capacitance retention (up to 90%).Increased performance energy storage devices with high areal capacity and outstanding adaptability are in high demand because to the rapid development of portable and wearable electronics. In order to prevent the Ti_3C_2 nanosheets from stacking again, amorphous FeOOH QDs have been created and synthesised into a hybrid film using just electrostatic repulsion. These QDs also contribute significantly to the capacitance of the film itself. The Ti_3C_2/FeOOH QDs hybrid film has excellent cycle stability (94.8% retention over 5000 cycles) in neutral electrolyte and 2.3-times higher areal capacitance (485 mF/cm^2) than the pure Ti_3C_2 film [41]. An asymmetric device, with the hybrid film serving as the negative electrode and MnO_2 on carbon cloth serving as the positive, has been constructed and functions at an output potential difference of 1.6 V. The device maintains 82% of its capacitance after 3000 cycles while providing a maximum energy density of 40 W h/cm^2 and a maximum power density of 8.2 mW/cm^2. Good capacitance and high flexibility are demonstrated by a wet-spun fibre made of $Ti_3C_2T_x$ nanosheets/$Ti_3C_2T_x$ quantum dots/RGO (reduced graphene oxide) (M6M3RG1). Capacitance and pliability of the M6M3RG1 fibre electrode are dose-dependent, and it has a new network topology with a maximum volumetric capacitance of 542 F cm-3. Injecting a homogenous solution of Ti3C2Tx nanosheets and PEDOT:PSS into a bath of 98 wt% H_2SO_4 is how the Ti_3C_2Tx/PEDOT:PSS [poly(3,4-ethylenedioxythiophene):poly(styrenesulfonate)] fibre (M7P3) is made

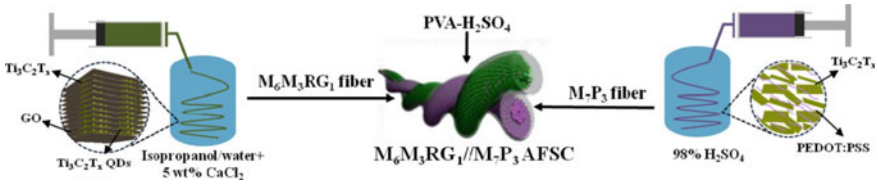

Fig. 2 Preparation schematic diagram of all-solid-state asymmetric fiber supercapacitors. Reproduced with the permission from Ref. [42] © 2020 American Chemical Society

(Fig. 2). A M6M3RG1//M7P3 asymmetric, flexible, solid-state supercapacitor is built in a PVA-H2SO4 gel electrolyte, with the M6M3RG1 fibre serving as the positive electrode and the M7P3 fibre serving as the negative electrode [42].

Next-generation asymmetric supercapacitor devices rely heavily on the advancement of high-performance anode materials. Using a simple two-step technique, a hollow and porous hierarchical anode of bismuth oxide/nitrogen-doped carbon dots (Bi_2O_3/NCDs) was constructed [43]. NCDs are shown to significantly enhance the specific capacitances of Bi_2O_3 porous nanosheets assembled into hollow microspheres, as well as stimulate their assembly. The improved specific capacitance of the Bi_2O_3/NCDs hierarchies is around 1046 F/g at a current density of 1 A/g, which can be attributed to the fascinating features of NCDs and the distinctive structure of Bi_2O_3/NCDs composites. The Bi_2O_3/NCDs hierarchical microspheres were used as the anode and the Ni(OH)2/NCDs nanosheets were used as the cathode in an asymmetric supercapacitor that displays an ultrahigh energy density of about 79.9 Wh/kg at 770.9 W/kg. Xia et al. developed a binder-free supercapacitor cathode electrode by directly growing $MnCO_3$ quantum dots (QDs, 1.2 nm) on $Ni(HCO_3)_2$-$MnCO_3$ hedgehog-like shell@needle composites onto a 3D macro-porous nickel foam [44]. This process was simple and scalable. Maximum specific capacitance of 2641.3 F/g at 3 A/g and 1493.3 F/g at 15 A/g was demonstrated by the $MnCO_3$ QDs/NiH-Mn-CO_3 composite electrode. More so than most reported nickel or manganese oxide/hydroxide-based asymmetric supercapacitors, the $MnCO_3$ QDs/NiH-Mn-CO_3 composites as the positive electrode and graphene as the negative electrode showed an energy density of 58.1 W h/kg at a power density of 900 W/kg and excellent cycling stability with 91.3% retention after 10,000 cycles. Energy storage devices should benefit greatly from using nickel molybdate ($NiMoO_4$), a ternary metal oxide. $NiMoO_4$ electrode has low electrical conductivity, which limits its usefulness [45]. Using a "dipping and drying" technique, silver quantum dots (Ag QDs/$NiMoO_4$) are deposited on the surface of mesoporous $NiMoO_4$ microspheres in yet another study. It's fascinating to see that the AgQDs and $NiMoO_4$ microsphere form an ohmic contact interface (electron anti-barrier layer). The composite electrode exhibits high specific capacitance (3342.7 F/g at 1 mV/s), good rate capability and improved cycling stability thanks to the Ag QDs/$NiMoO_4$ microspheres' high specific surface area, abundant surface active sites, enhanced interfacial conductivity, and porous structure.

2.3 QDs in Hybrid Ion Capacitors (HIC)

In the exciting new field of Hybrid Ion Capacitors (HIC), which combines the best features of batteries and supercapacitors, QDs play a pivotal role? Energy density, charge/discharge speed, and cycle life are all areas where HICs excel above more traditional forms of energy storage. There is hope for improving HIC efficiency by including QDs into design. Quantum dots are semiconductor particles on the nanoscale scale that exhibit unusual electrical and optical characteristics. HICs benefit from increased ion storage capacity and streamlined charge transfer when QDs are incorporated into the electrode materials. This is because QDs have a high surface area and can have their electrical characteristics tuned. Because of this, HICs can store more energy and quickly release it, making them perfect for applications that need power bursts. In addition, QDs can be made to display tailored energy levels, allowing for granular regulation of charge storage methods. By tackling the primary obstacles associated with energy storage, such as short cycle life and sluggish charge/discharge rates, this custom design improves the overall efficiency and durability of HICs. One promising path towards the creation of high-performance energy storage devices is the use of quantum dots in hybrid ion capacitors. By providing solutions that are efficient, long-lasting, and high-energy–density, their integration has the potential to revolutionise many different applications, such as portable devices, electric cars, and grid-level energy storage.

Wu et al. manufacture an innovative SC architecture (ASC) in the shape of an accordion, complete with a hierarchical porous framework [46]. Pseudocapacitive K^+ storage is considerably enhanced by the ASC's highly exposed structure following electro-deposition of extremely electrochemically active nitrogen-doped graphene quantum dots (N-GQDs). Sodium ion hybrid capacitors (SIHCs) are severely limited in their performance due to the kinetics incompatibility between their battery-type anode and capacitive-type cathode. In a first of its kind study, researchers created a SIHCs device by connecting freestanding anode quantum grade vanadium nitride (VN) nanodots (Fig. 3) anchored in one-dimensional N/F co-doped carbon nanofiber cages (VNQDs@PCNFs-N/F) with corresponding activated N/F co-doped carbon nanofiber cages (APCNFs-N/F) as cathode [47]. To overcome this kinetics mismatch, quick sodium storage can be ensured by combining VN quantum dots with N/F co-doped 1D conductive carbon cages, which strongly couples the two materials together. Density functional theory simulations further show that the high concentration of active sites in the VNQDs@PCNFs-N/F configuration is beneficial for enhancing Na + adsorption/reaction activity, which in turn boosts "intrinsic" and "extrinsic" pseudocapacitance and the kinetics of the anode.

The sodium ion hybrid supercapacitor (Na-HSC) is gaining popularity as a form of energy storage because to its high power density and long cycle life. The advancement of this technology relies on the use of anode materials with a high rate of Na^+ embedding. In this study, we used yeast enriched with Nb^{5+} and a straightforward hydrothermal-carbonization method to create multi-chamber microspheres with Nb_2O_5 quantum dots (T-Nb_2O_5@YC). The electrochemical performance of

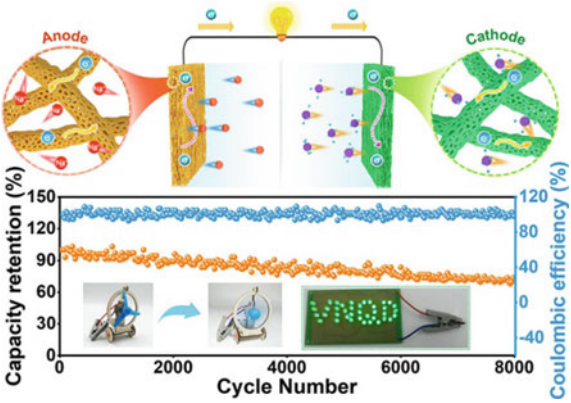

Fig. 3 Schematic illustration of the assembled VNQDs@PCNFs-N/F||APCNFs-N/F device. long-term cycling stability at 1 A g–1 for the VNQDs@PCNFs-N/F||APCNFs-N/F device. Inset: digital pictures of the LEDs and wind trolley powered by our device. Reproduced with the permission from Ref. [47] © 2022 American Chemical Society

Na+ half-cells and Na-HSCs is excellent [48]. Using an easy thermal synthesis process and some old cola soda, a multi-gram quantity of water-soluble, fluorescent carbon nanodots (C-Dots) were synthesised and used as a conductive and ion-donating component in a supercapacitor. The quantum yield of these C-Dots varies from 4.6 to 18.3%. The size, crystal structure, morphology, and surface properties of C-Dots were examined and compared using fluorescence and electrical properties, as were their Raman and electron paramagnetic resonance spectra. Nanorods of polypyrrole (PPy) doped with manganese dioxide (MnO) and adorned with carbon dots (C-Dots) were used as anode material in a supercapacitor experiment. Cathode materials comprised of reduced graphene oxide were doped with a dicationic bis-imidazolium-based ionic liquid to improve charge transfer and surface wetting. Octyl-bis(3-methylimidazolium) diiodide (C8H16BImI), synthesised by N-alkylation process, was used as the electrolyte in the liquid ionic membrane [49]. Research using paramagnetism and impedance spectroscopy has been conducted to trace the hybrid capacitor's performance back to its roots. In particular, the quality of the synthesis, as well as the selection of electrode and electrolyte materials, all contributed to the extraordinarily high capacitance value ($C = 17.3\ F/cm^2$) we observed. Li-ion capacitors (LICs) with battery-type anodes and capacitive cathodes could be the next generation of electrochemical energy storage (EES) devices. This is because they have a high energy density (ED) and a high power density (PD), which is mainly determined by the chemical and physical properties of their anodes and cathodes. We used a one-step solvothermal method to coat $Ti_3C_2T_x$ MXene sheets with SnO_2 quantum dots (QDs, about 4–5 nm) that have oxygen gaps on their surface [50]. The sheets had different amounts of SnO_2 quantum dots. The improved SnO_2 QDs/$Ti_3C_2T_{x\text{-}50}$ composite anode has a high reversible discharge capacity (1000.4 mAh/g@0.2 A/g), excellent cycling stability (the discharge capacity of 1004 mAh/g at the end of 300 cycles at the same current density), and good rate performance. The reaction kinetics is dominated by the fast capacitive process to a large extent, because SnO_2 QDs has fewer Li^+ ions and oxygen vacancies.

3 Conclusions and Summary

It is clear that QDs-based electrode materials have enormous potential in the design of electrode materials in supercapacitors due to their unique quantum size, ease of functionalization on the surface, and abundance of functional groups. In modern supercapacitors, QDs are utilised primarily to alter the electrode materials through the introduction of supplementary chemicals or QDs-derived carbon materials created through the high-temperature treatment of QDs precursors. Keep in mind that the QDs' low electron conductivity and limited BET area mean that they won't provide good electrochemical performance if utilised directly as electrode materials.

QDs have been successfully integrated into supercapacitors, suggesting a promising future for these energy storage devices. Supercapacitors can be made more effective by taking use of QDs' high surface area, customizable electronic characteristics, and efficient charge transfer. Improved energy density, faster charge/discharge rates, and greater long-term stability are the primary benefits of employing QDs in supercapacitors. Several promising future directions for QDs in supercapacitors are emerging. Developing new QD materials and unique electrode designs are two areas where researchers are currently looking for methods to improve QD-based supercapacitors. This has the potential to lead to even higher energy densities and longer cycle life, expanding the applicability of QD-enhanced supercapacitors to uses beyond just renewable energy storage and portable devices, such as electric vehicles. Making these cutting-edge supercapacitors more easily available and environmentally benign through the use of sustainable and inexpensive QD materials is also a continuing priority. Scaling up production processes and improving manufacturing methods are also crucial for bringing QD-based supercapacitors to market. To sum up, Quantum Dots may herald a new era in energy storage technologies and could cause a revolution in the supercapacitor business. We can expect more sustainable and energy-efficient supercapacitors to play a role in the future if research and development efforts are maintained.

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Quantum Dots Composites for Energy Storage Applications

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Abstract The demand for developing energy conversion and storage systems is increasingly growing due to the escalating environmental problems effects caused by global warming and fossil fuels depletion. The rapid expansion of renewable energy sources such as solar energy, wind energy, biofuels, hydropower, geothermal, and hydrogen energy is expected to be critical to alleviating the energy crisis. Quantum dots (QDs) are nanocrystalline semiconducting materials with a few nanometers in diameter, ranging from 1 to 10 nm. Their electrical and optical characteristics can be manipulated by altering the size up to 10 nm, which is attributed to the quantum confinement effect. However, the size of the quantum dots depends on the semiconducting material because the exciton Bohr radius varies depending on the type of semiconductor. Many literature articles have highlighted the application of quantum dots in supercapacitors (SCs), metal-ion batteries (MIBs), and photocatalytic and electrocatalytic hydrogen evolution. This chapter is dedicated to presenting recent progress regarding the potential application of quantum dots and related composite materials in various energy storage systems.

Keywords Quantum dots · Quantum confinement effect · Li-ion batteries · Li-Metal batteries · Supercapacitors · Hydrogen storage

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Abbreviations

QDs	Quantum dots
SCs	Supercapacitors
MIBs	Metal-ion batteries
0D	Zero-dimensional
SCQDs	Semiconductor QDs
GO	Graphene oxide
CNTs	Carbon nanotubes
CDs-QDs	Cadmium sulfide QDs
CQDs	Carbon quantum dots
LIBs	Lithium-ion batteries
SEI	Solid electrolyte interface
SIBs	Sodium ion batteries
GQDs	Graphene quantum dots
GO	Graphene oxide
SSA	Specific surface area
rGO	Reduced graphene oxide
SWCNTs	Single walled carbon
MNC	Multilayered N-doped nano carbon sheets
BP	Black phosphorous
RP	Red phosphorus
MOFs	Metal organic frameworks
HCP	Hollow carbon polyhedral matrix
PIBs	Potassium-ion batteries
LMBs	Li-metal batteries
LVCF	Lithophilic vertical cactus-like framework
PEO	Polyethylene oxide
LSBs	Lithium-sulfur batteries
CTAB	Cetyltrimethylammonium bromide
PEI	Polyethyleneimine functionalized
CB	Carbon black
OER	Oxygen evolution reaction
ORR	Oxygen reduction reaction
ZIBs	Zinc-ion batteries
ZMO	$ZnMn_2O_4$
EDLC	Electric double-layer capacitors
PCN	Porous carbon nanosheet
ASCs	Asymmetric super capacitors
AC	Activated carbon
ZIF-8	Zeolitic imidazolate framework
FGS	Functionalized graphene nanosheets
HICs	Hybrid super capacitors
MSCs	Micro super capacitors

NF Ni foam

1 Introduction

Decarbonized green energy, including solar, wind, hydro, and geothermal energy, is the solution to the global warming issue, the crisis of energy, and harmful emissions to the environment [1]. In terms of carbon dioxide neutrality, new sources of energy will take over as the main source of electricity, and storing massive amounts of renewable energy will be an immense challenge [2]. According to their intermittent nature, these energy sources should be stored and released when necessary [3]. Electrochemical energy storage represents a promising solution to such a worldwide challenge. Supercapacitors and batteries are suitable examples for these types of storage technologies [4]. Furthermore, encouraging the creation of electrochemical hydrides and photochemical bonding to produce fuel is the foundation of photocatalytic hydrogen evolution, another "clean" method of energy storage [5].

Quantum dots have attracted a lot of research interest recently due to their potential electrochemical energy storage because of their huge specific surface area, controllable size, far-short ion/electron transport channel, non-toxicity, cost-effectiveness, adaptable photoluminescence, and simple functionalization of the surface [6]. Environmental problems are further exacerbated by the usage of fossil fuels and global warming. The field of quantum dots (QDs) is rapidly advancing in terms of energy conversion and storage. Less than 10 nm in diameter, sphere- or quasi-sphere-shaped materials make up the majority of QDs [7]. Synthesis methods of QDs are typically divided into two main categories: "top-down" and "bottom-up" techniques [8]. The top-down approach often begins with a massive amount of materials and progresses to QDs by a variety of techniques, including chemical etching, microwave-assisted irradiation, ultrasonication processing, hydrothermal/solvothermal technique, electrochemical method, and some others [9]. A bottom-up synthesis method can also be used to chemically develop QDs from tiny organic and inorganic precursors [10]. Numerous methods have been used to create QDs, which are zero-dimension materials with relatively large bandgap, extremely small size, and high surface to volume ratio [11]. These materials exhibit unusual physicochemical properties because of their strong quantum confinement and edge effect. During the synthesis of QDs, their structure, size, and heteroatomic functional groups can simply be controlled. As a result, they have more active sites per unit mass, are more physically and chemically tunable, and are more able to hybridize with other nanomaterials [12].

An electrochemical energy storage system's overall efficiency is greatly influenced by the electrode materials used [13]. Heteroatomic functional groups abound on the surface of QDs, providing a wide range of active sites. Considering their superior conductivity of ions, rapid rate of charge–discharge, high capacity, cycle stability, and other characteristics, they can be used in composites for current collectors [14] and active material electrodes [15]. This would significantly improve the

quality of electrochemical energy storage systems. Because of their distinct features as electron donors and acceptors as well as their excellent electron transport properties, QDs are an extremely stable and effective photocatalyst for processes involving the generation and storage of hydrogen and the reduction of oxygen [16].

2 Types of QDs for Energy Storage Applications

QDs are zero-dimensional(0D) materials with less than 10 nm in diameter. They are spherical or quasi-spherical morphologies that have attracted a lot of researchers' interest in the context of energy storage and conversion. They can be synthesized using a variety of preparation techniques, such as the chemical method, by assembling the tiny organic and inorganic precursors [17]. These techniques aim to tune physicochemical properties of the materials by adjusting the bandgap that emerges from the strong quantum confinement and controlling the material dimensions to be 0D with ultra-small sizes. In addition to short ion/electron transfer paths, high dispersibility, and variable surface functionalities [18]. The QDs are classified into various types have been using in the energy storage applications as follow.

2.1 Semiconductor QDs (SCQDS)

The remarkable optical characteristics of SCQDs such as their strong photo bleaching resistance and narrow emission band with broad excitation, have recently attracted a lot of attention [19]. Additionally, they have been used in various applications such as biomedical sensors [20], biological cell templates [21], biomolecular imaging [22], bioconjugation [23], solar cells [24], light-emitting diodes [25] and catalysis [26]**.** Another area where QDs have received little attention is electrochemical energy storage [27]. This is mainly due to the difficulties in producing large amount of stable and monodispersed QDs [28]. To overcome these limitations, specific chemical procedures are used, such as digestive ripening, to create monodisperse QDs from polydisperse particles [29]. Therefore, QD-based materials have the capability to be utilized in electrochemical energy storage applications as a result of their distinctive properties, including their high surface area, electroactive sites, exceptional electrical conductivity, and short ion diffusion length [30]. Additionally, because of their very comparable surface chemistries, their bulk behavior is probably more predictable, which has the benefits of high yield and manufacturability.

The charge storage properties of QDs in terms of capacitance and cycle stability are improved by integrating them with carbonaceous materials like graphene oxide (GO) and carbon nanotubes (CNTs), which results in uniform distribution of the QDs on the surfaces of the GO and CNTs and prevents agglomeration of the QDs, as well as increasing the conductivity of the nanocomposite [31]. Few research studies have been performed on the SCQDs for energy storage applications such as supporting the

cadmium sulfide QDs (CdS-QDs) onto the surface of carbon nanotubes to be involved in lithium-sulfur batteries as the sulfur cathode [5], using CdSe/ZnS QDs@CNTs composite for rechargeable photo-electrochemical Li-O_2 batteries [32].

2.2 Metal Oxide QDs

Metal oxide nanomaterials, particularly transition metal oxide QDs, are considered as a prominent popular material for lithium-ion batteries (LIBs) and SCs because of their acceptable cost, natural availability, non-toxicity, and excellent safety. Due to their great density, they can attain high volumetric performance [33]. Furthermore, their specific capacity is usually two to three times higher when compared to their carbon-based counterparts [34]. Nevertheless, they demonstrate reduced overall performance and cycle stability values due to their low electrical conductivity, which restricts their utility in energy storage, so a lot of research studies have been performed to enhance their electrochemical characteristics such as electrical conductivity, cycling stability, and rate performance by decreasing their sizes to the nanoscale and simultaneously integrating with carbonaceous materials [35].

The carbonaceous materials, such as nitrogen-doped graphene, CNT, and CQDs, could prevent the agglomeration of the metal oxide nanoparticles [36], improve the attachment of the metal oxides such as TiO_2, Fe_3O_4, CoO, CuO, and SnO_2 on the carbon surfaces, as well as, increase the electroactive sites [37]. This results in fast charge transfer, good wettability at the solid electrolyte interface (SEI), and high conductivity of electricity. All these outstanding properties give high potential for the metal oxides to be effective electrodes for LIBs or sodium-ion batteries (SIBs) [38].

2.3 Graphene Quantum Dots (GQDs)

GQDs are a relatively recent finding within the allotropic carbonaceous family, which also includes graphite, diamond, nanotubes, fullerene, and graphene. They combine a number of advantageous properties of conventional SCQDs without the drawbacks of elemental shortage or inherent toxicity, as well as requiring labor-intensive, expensive, or ineffective preparation procedures [39]. GQDs are functionalized nanoscale pieces of graphene, typically less than 10 nm in size. Their lateral dimension being greater than their height is the source of their anisotropic morphology.

GQDs can be prepared through two synthetic approaches, each of which relies on either top-down or bottom-up synthetic processes. In brief, top-down synthesis method involves degradation of large carbon source such as carbon fibres, GO, coal, fullerenes, graphite into smaller fragments using concentrated [40]. Because graphene oxide (GO) has many functional groups that contain oxygen atoms which promote chemical cleavage towards nanosized GQDs, GO is often the best starting

material. However, unlike graphite, GO is not a naturally occurring substance; rather, it must first be produced by Hummer using a complicated chemical process using a range of materials, including anthracite or coal. The use of graphite as a natural source has been investigated as a potential alternative to GO but has so far had less success due to lower synthetic yields. The common methods for preparing the GQDs are microwave-assisted, hydrothermal or solvothermal, and electrochemical methods. Contrarily, bottom-up approaches based on organic precursors carbonization like citric acid, glucose, and others have poorer yields and difficulty purifying from the unreacted tiny organic components [38].

Despite numerous attempts, conducting studies on the fabrication of GQDs-based various nanomaterials to expand their application in the energy-related domains is still in its early phases. There has been a persistent drive among researchers in this context to direct their attention to the innovative and reasonable fabrication of GQDs-based nanomaterials connected to electrochemical energy storage and electrocatalysis. Consequently, a rapid expansion of new active materials based-GQDs research and its possible applications in the energy sector is anticipated in the near future. As a cutting-edge material for electrodes in electrochemical energy storage devices like batteries and supercapacitors, GQDs have been investigated. For improving the physicochemical properties of the GQDs, they could be integrated with graphene nanosheets to combine the advantages of both materials and give them a significant promise in a variety of catalytic and energy storage applications [41].

2.4 Carbon QDs

Carbon dots attracted great attention as a new derivative member of the carbon family with diameters not larger than 10 nm [42]. They have sp^2 graphitic core structure with oxidized functionalities. Since carbon dots have semiconducting characteristics, they can substitute SCQDs in optoelectronic devices [43]. Carbon dots have great potential for a variety of real-world applications, including photocatalysis, solar cells, bio-imaging, and bio-sensing, because of their exceptional characteristics including huge luminescence, high solubility, biocompatibility, and excellent chemical stability [44]. Additionally, carbon dots have a potential future as next-generation energy storage materials. Since 2013, the number of studies on carbon dots-based electrode materials has increased dramatically.

Early on, a few innovative research teams made some promising advancements by utilizing carbon dots to create electrode active materials for different energy storage systems like MIBs [45], metal-S batteries [46] and supercapacitors [13]. Carbon dots have revealed advantages when used as active electrode materials since they have been improving some characteristics in MIBs, supercapacitors, metal-air batteries such as coulombic efficiency, long cycling life, functional groups, and interfacial wettability at the SEI [26]. Furthermore, integrating the carbon dots in nanocomposites to be involved as electrode active material in SCs results in enhanced

electron transport and ionic motion at the SEI, fast faradic reactions, and high specific capacity [41].

3 QDs Based Nanocomposites for Energy Storage Applications

Electrochemical devices to store energy including batteries and supercapacitors serve as the primary solution for addressing the challenge of intermittent energy sources found in nature, so energy can be stored and released later. Recently, QDs and their composites have been widely employed in the field of electrochemical energy storage because of their distinctive characteristics, including a significant specific surface area (SSA), tunable size ranging from 2–10 nm, low cost, non-toxicity, short ion diffusion distance, facile surface functionalization [6]. QDs are synthesized via several methods to become zero-dimensional materials showing unique physicochemical properties based on the strong quantum confinement effect resulting in a relatively large band gap and a considerable surface area-to-volume ratio [35]. The heteroatomic parameters that can be regulated during their synthesis enhance the surface of QDs, allowing for a diverse array of active sites, the ability to tune their physicochemical properties, and the potential for hybridization with other nanomaterials, which greatly provides more flexibility and adaptability during their usage [47]. QDs based composites can be used as a current collector or electrode active materials [48], obtaining superior conductivity of ions, rapid rate of charge–discharge, short ion diffusion length, high capacity, cycle stability, and other characteristics. Another efficient approach to storing energy is through photo-electrocatalytic hydrogen generation that involves forming electrochemical hydrides and bonding them photochemically to create fuel [4].

QDs show special effects during charge–discharge processes such as the low charge transfer resistance, short ion diffusion distance, superior surface characteristics, quantum confinement effect, and QDs structure against volume change [48]. Each of which has received great attention in the field of energy storage. QDs with smaller sizes demonstrate reduced charge transfer resistance and excellent electrical conductivity, which leads to a relatively high capacity of batteries. Wang et al. fabricated ZnS QDs/N-doped reduced graphene oxide (rGO) composite for Li-CO_2 battery which has an extremely low charge transfer resistance when compared to other cathodes without QDs [49]. The ultrasmall size of QDs can efficiently shorten the ion diffusion length, thereby facilitating rapid charge transfer during electrochemical reactions. Cheng et al. reported N-doped carbon decorated with SnO_2 QDs which in turn shortened the ion diffusion distance with superior performance in LIBs and SIBs [50]. The noticeable factor of QDs is the enhanced active surface area and hence, high percentage of edge atoms that improves the supercapacitor's ability to store charges and boosts ion adsorption or desorption activity. Bonu el al. investigated SnO_2 QDs with a substantial surface area-to-volume ratio as a supercapacitor active material.

The constructed supercapacitor possessed good activity, high conductivity with reinforced specific capacity, and no volume variation during the electrochemical process in comparison to other nanoparticles [51]. The quantum confinement phenomenon exhibited by QDs can enhance their electrical conductivity, thereby contributing to the improvement in the performance of energy storage systems. Zhou et al. synthesized Co_3O_4 QDs/graphene composite via a facile microwave irradiation process which was used as anode material for LIBs and possessed clearly improved cycling performance, and high-efficient charge storage kinetics [52]. Liu et al. fabricated hybrids of graphene-Fe_3O_4 QDs as anode materials in SIBs. The hierarchical structure of the obtained G-Fe_3O_4 QDs composite showed superior storage capacity of sodium ions and considerably enhanced its cycling stability [53]. Different types of reported QDs used in different energy storage devices including rechargeable batteries, supercapacitors, and storage of hydrogen will be illustrated in the subsections below in more detail.

3.1 QDs for Rechargeable Batteries

QDs are employed as electrode materials in rechargeable batteries for their beneficial properties towards enhancing capacity, rate capability, conductivity, and cycling stability [54]. Herein, we discuss different QDs based nanocomposites where one or more of the composite components are in the form of QDs for diverse types of batteries.

3.1.1 QDs in Metal Ion Batteries (MIBs)

MIBs storage entails a charge–discharge process commonly referred to as a "rocking chair" mechanism, where the alkali-metal ions (such as Li, Na, and K-ion) move from the cathode to the anode during charging and return during discharging. MIBs have an edge over conventional batteries in terms of extended lifespan, greater energy density, with no memory effect. Nevertheless, MIBs encounter issues related to unstable performance and low power density. Several studies have investigated the utility of QDs-based composites as anode materials in MIBs that can significantly improve the electrochemical capabilities [55].

Lithium-Ion Batteries

Several metal oxide QDs based nanocomposites are used as anode materials for LIBs. ZnO possesses higher electrical conductivity, theoretical capacity of about 978 mA h/g, more abundant than other metal oxide anodes which make it suitable as a LIB anode [56], however, ZnO is rather scarce because of its poor cycle stability. The process by which ZnO stores charge involves a process of conversion

with Li^+, before alloying to generate LiZn, consequently resulting in a 228% volume change, which in turn causes the anode to pulverize and a rapid fading of capacity. Thus, ZnO anode needs to be controlled below a critical size with enhanced flexibility to shield the volume change [57]. Sun et al. reported ZnO QDs/Graphene sheets with tunable sizes of 2, 4, and 7 nm. The tension resulting from the change in volume of ZnO QDs is limited by the great mechanical stability of graphene. ZnO QDs are strongly anchored on graphene, which keeps them from clumping together during cycling and creates an extremely stable anode. The optimum electrochemical activity of ZnO QDs is represented by confining their size to about 2 nm with a specific capacity of 560 mA h/g at100 mA/g current [56]. Tan et al. prepared ZnO/rGO composite electrode which achieved outstanding performance in anodes of LIBs [58]. ZnO QDs contained by amorphous carbon have some advantages over crystalline materials, primarily because of the isotropic insertion of Li^+ ions therein, which is more susceptible to structural strain in crystalline materials. Ma et al. implanted ZnO QDs in a matrix of N-doped porous carbon (ZnO@NPC) on a single walled carbon nanotubes (SWCNTs) film. The obtained spongy film of ZnO@NPC/SWCNTs was used as a self-standing and binder-free anode which exhibited a highly stable cycling stability [59]. ZnS QDs were uniformly distributed in multilayered N-doped nano carbon sheets (ZnS QDs@mNC) in a tailored architecture created by Fang et al. This allowed for ZnS QDs expansion in volume during cycling with just a 6.5% total volume expansion [60]. Zhang et al. used an in-situ ion exchange technique to grow ZnO@ZnO QDs/C nanorod clusters on conducting carbon cloth. Because of the synthetic composite nanorod arrays' better structure stability, good electron accumulation efficiency, and reduced Li-ion diffusion length, they demonstrated excellent rate capability and cycle stability [61].

TiO_2 has been investigated for anodes of LIBs. TiO_2 shows poor electronic conductivity that restricts the mobility of Li-ion within TiO_2 crystal. Thus downsizing the TiO_2 to QDs and inserting them in other conductive surfaces have speeded up the reaction kinetics and enhanced the total electrochemical efficacy. Mo et al. synthesized TiO_2 QDs/G composite as an anode active material in LIBs. TiO_2 QDs showed well dispersion on graphene substrates with no aggregation in addition to preventing the stacking of the nanostructure with cycling [62]. Meng et al. reported black phosphorous (BP) QDs/Ti_3C_2 nanosheets as anodes with a dual mechanism of storing energy in LIBs [125]. BP QDs decorated on the Ti_3C_2 nanosheets exhibited of about 910 mA h/g at 100 mA/g, and excellent 100% cycle retention reserved after 2400 cycles. By using a ball-milling technique, Zhang et al. synthesized a unique red phosphorous (RP) QDs/$Ti_3C_2T_x$ composite that demonstrated outstanding electrochemical behavior in LIBs. Because of their intimate attachment to the MXene sheets, the RP QDs improved Li-ion transfer throughout cycling operations [63]. Lee et al. synthesized ZnS quantum dots embedded in N-doped carbon matrix as and employed it in LIBs as shown in Fig. 1 [64]. The ZnS-QD@NC electrode has a reversible specific capacity of 800 $mAhg^{-1}$, a high initial energy round-trip efficiency of 83%, and higher cycling stability and power due to the composite's structural properties and unique electrochemical activity.

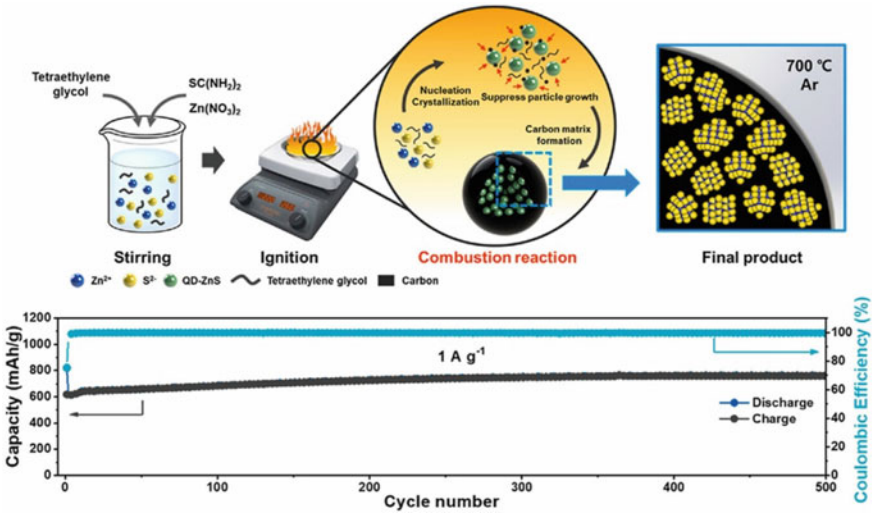

Fig. 1 A schematic illustration of ZnS-QD@N-doped Carbon for Li-ion battery anode, reproduced with permission Copyright (2022) [64]

Various studies concerned with SnO_2 or Sn-dichalcogenides QDs based composites in LIBs to address the challenges of reduced conductivity, high volume expansion > 300% of bulk SnX_2 (X = O, S, Se) during the Li inserting-deinserting process. Carbonaceous substrates are the most suitable conducting hosts of SnX_2 QDs. Zhao et al. demonstrated SnO_2 QDs/rGO by a simple hydrothermal approach where an increased level of oxidation of rGO simplifies the consistent dispersion of QDs [63]. Gao et al. have developed a 3D structure with a flexible network of SnO_2QDs/ negatively charged GO with exceptional flexibility, which could be utilized as a binder-free electrode due to the strong chemical adhesion of SnO_2 QDs to GO surface [65]. Li et al. synthesized a novel composite of SnO_x QDs loaded onto the Zr-metal organic frameworks' (MOFs) surface. During the alloying and dealloying process, SnO_x QDs@Zr-MOFs offers surrounding room to accommodate the volume change of SnOx QDs [66]. Huang et al. stated that $SnSe_2$ QDs/rGO nanocomposite possessed high reversible capacity of 778.5 mA h/g at 50 mA/g, remarkable rate capacity of 324.5 mA h g^{-1} at 5 A g^{-1} current and high cycle retention of 92.2% after 3000 cycle at 5 A g^{-1} [67]. Other metal oxide QDs based carbonaceous materials such as Co_3O_4 QDs, CoO QDs, Fe_3O_4@C QDs, MnO QDs, V_2O_5 QDs are employed as anode materials for LIBs [68].

GQDs, graphitic-type CQDs are most preferred in the surface functionalization of metal oxide cathode active materials. Balogun et al. reported a 3D carbon cloth with VO_2 interlaced nanowires decorated with CQDs. This structure reduced lithium-ion diffusion length and offered 86% as an overall capacity of LIB [69]. Xuewei Zhao et al. have synthesized GQDs@CNTs as a hybrid structural electrodeas illustrated in Fig. 2. The functionalized 3D CNTs sponge with GQDs improved the electrochemical

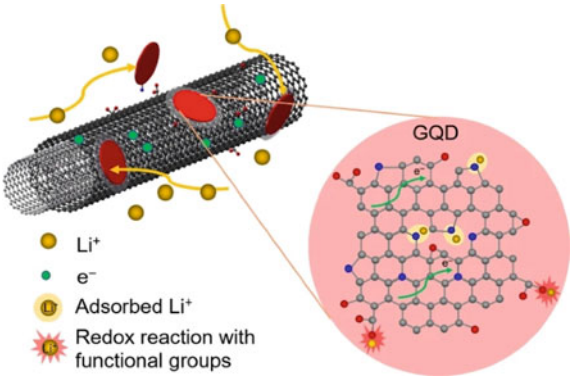

Fig. 2 Synthesis of GQD@CNTs hybrid sponge reproduced with permission Copyright (2020) from Springer Nature [70]

performance [70]. Yingchang et al. have prepared N-doped TiO_2/CQDs that offered 93.6% cycling stability of retention after the 300th cycle [71].

Sodium-Ion Batteries

Rechargeable SIBs are among the most affordable alternatives to LIBs due to the availability of sodium and high theoretical capacity of 1165 mA h g^{-1}. Nevertheless, SIBs feature substantial volume expansion, struggling conductivity, and Na-ion diffusion kinetics. Hence, several studies were performed to overcome the forementioned barriers. Carbonaceous materials functionalized with different QDs are the most favorable alternatives to be used as SIB anodes [58].

Yu et al. synthesized a composite of TiO_2 QDs and rGO to serve as an anode for SIBs. Superior mechanical stability and pathways for the passage of electrons and ions were made possible by the porous structure [72]. Liu et al. synthesized graphene-Fe_3O_4 QDs to serve as an anode active material for SIBs via a hydrothermal/solvothermal technique. Fe_3O_4 QDs were homogenously loaded on a 3D graphene sponge demonstrating high sodium-ion storage capacity of 525 mA h/g at 30 mA/g, good cycling stability of 312 mA h/g after 200 cycles at 50 mA/g, and high-rate performance [52]. Kong et al. reported Fe_3O_4 QDs/MoS_2 nanosheets on graphite flakes with outstanding reversible capacity and good rate performance [73]. Zhang et al. prepared a composite of ZnS QDs/graphene as anode via a one-pot hydrothermal method. This ZnS QDs/G composite demonstrated an adjustable specific capacity of 491 mA h/g at 100 mA/g after the 100th cycle [74]. Wang et al. synthesized SnO_2 QDs/G aerogel nanocomposite which demonstrated better characteristics as anode active materials in SIBs than individual SnO_2 [75].

ZnS Metal sulfide QDs are also of high importance as anode materials for SIBs. Co_9S_8-QDs@NC/Na_3V_2 $(PO_4)_3$ were tested and gave the composites advantages including an exceptional rate capability, a high reversible specific capacity, and outstanding stability [76]. Furthermore, SnS, SnS_2, and ZnS QDs were used as possible anode materials for high-performance SIBs [77]. Chen et al. reported a

dual strategy confining Co_9S_8 QDs, where the Co_9S_8 QDs were involved within a mesoporous hollow carbon polyhedral matrix (HCP), and HCP was also embedded in a macroporous rGO structure forming a sponge-like architecture. The obtained Co_9S_8-QDs@HCP/rGO structure caused a significant capacity of 72% at a scan rate of 0.6 mV/s, offering high-power performance electrodes [78].

Carbonaceous materials are promising candidates for SIB anode. Liu et al. developed graded $Na_3V_2(PO_4)_2F_3$ CQD microspheres for SIB cathode with high capacitive capabilities and acceptable rate performance [55].

Potassium-Ion Batteries (PIBs)

PIBs are another promising and attractive energy storage alternative regarding their cost effectiveness and availability. Even though PIBs have a high potassium ionic radius (1.38 Å) that causes them to function inadequately, reducing the active material's size to the QD scale provides a practical way to address the main electrode barrier issues. Gao et al. constructed a CoS QDs@graphene composite via a facile two-step solvothermal/hydrothermal method which offered fast ion/electron transfer capability, good structural stability, and excellent electrochemical performance [79]. Liu et al. fabricated $Co_{0.85}Se$ QDs encapsulated in mesoporous carbon that was used as anode and demonstrated remarkable K-ion storage performance because of its hierarchical structure [80]. Hussain et al. employed Co_3Se_4 QDs decorated with N-doped carbon (QDs@C) as anode for PIBs, which possessed excellent discharge capacity and good cyclic performance [81]. Moreover, constructing bimetallic sulfide QDs for anodes of PIBs can improve its cycle performance. Cao et al. synthesized $Cu_{12}Sb_4S_{13}$ QDs/Ti_3C_2 nanosheets (CAS-Ti_3C_2) as anode for promoting the K-ion storage [82]. Erjin et al. have prepared a 3D structure of CQDs on rGO. PIB with CQDs@rGO gives a remarkable capacity of 310 mA h/g at 0.1 A/g [83].

3.1.2 Quantum Dots in Lithium-Metal Batteries

It is highly desirable to build Li-metal batteries (LMBs) with high energy density due to their large theoretical capacity (3860 mA h/g) [84]. Unlike LIBs that employ intercalated graphite anodes, Pristine Li metal is involved as the anode to increase the energy density of LMBs. The deposited Li ions on the anode's surface form dendrites which can easily penetrate the separator leading to a short circuit. The randomly deposited Li ions causes volume expansion which restricts their practical application in various battery-types including LIBs, LMBs, LSBs, and Li-O_2 batteries as well. Hence, battery safety is of great consideration while studying LMBs [85]. To improve the functionality and safety of Li metal anodes, Liu et al. inserted ZnO QDs/CNTs in a lithiophilic vertical cactus-like framework (LVCF) derived from a Zn/Cu-based coordination polymer as shown in Fig. 3 [84].

Wang et al. prepared a composite of perovskite $CsPbI_3$ QDs with Polyethylene oxide (PEO) that notably improved the current properties and power capabilities by

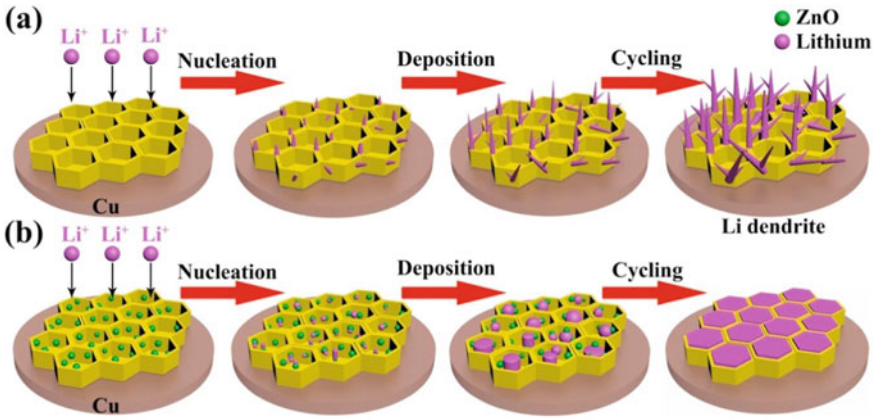

Fig. 3 **a** For hierarchical porous carbon (HPC) with no ZnO, **b** ZnO modified HPC scaffold with permission Copyright (2017) from Elsevier [84]

promoting better conductivity and a higher Li-ion transference number in LMBs. They have demonstrated that the presence of Lewis acid–base interactions plays a crucial role in facilitating the performance of PEO-Perovskite QD solid-state electrolytes. This phenomenon arises from the ability of these interactions to hinder the crystallization process of quantized $CsPbX_3$ and to promote robust electrostatic adsorption of halogen atoms [86].

3.1.3 QDs in Lithium-Sulfur Batteries

Rechargeable Lithium-Sulfur batteries (LSBs) possess a high theoretical capacity of 1675 mA h/g, high energy density 2600 Wh/kg which is 5 times higher than conventional Li-ion counterparts in addition to their reasonable cost [87]. QDs can be engaged as the active material of cathodes and separators in LSBs [88]. Nevertheless, there are still several issues with LSB commercialization, namely the shuttle effect and sluggish reaction kinetics that cause rapid capacity loss during discharge.

Several studies concerned the modification of sulfur cathodes of LSBs using QDs based nanocomposites. Gao et al. followed a facile strategy to synthesize TiO_2 QDs on thin MXene ($Ti_3C_2T_x$) nanosheets via hydrothermal method using cetyltrimethylammonium bromide (CTAB). The resulting TiO_2 QDs/MXene composite exhibits strong conductivity to guarantee quick ion diffusion and prevents the shuttle effect of polysulfide [89]. Hu et al. developed polyethyleneimine functionalized carbon quantum dots (PEI/CQDs) to enhance the performance of Li–S batteries that have high sulfur loading. Enhancement of Li-ion flow characteristics of polysulfides in the cathode with PEI/CQD modification [90]. Park et al. synthesized oxygenated GQDs from carbon fiber using a modified Hummers' technique, demonstrating that they possess good sulphidic characteristics. To create a homogeneous GQD/S coating

and a densely packed CB@GQDS@S composite with high structural and mechanical stability, GQDs/S was combined with carbon black (CB). This prevented the cathode from pulverizing during cycling and preserved the cathode's structural integrity [91]. Cai et al. successfully designed CdS QDs incorporated into CNTs to enable sulfur infiltration as the cathode material in LSBs. This unique configuration exhibited a strong ability to adsorb polysulfide and showcased exceptional performance at high rates. [62]. Moreover, CuS QDs, MoS_2 QDs [92], and ZnS QDs@rGO [93] are also referred as cathodes in LSBs.

Functional separators such as CQDs, numerous metal oxides/sulfides/carbides QDs, and even non-metallic like phosphorus QDs, have been involved in LSBs. Typically, these QDs are adorned on a carbonaceous substrate and employed as a sulfur host or to enable the separator to work [94]. In order to mitigate the shuttle effect and get rid of electrode expansion during the reaction process, Ding et al. created a separator of TiO_2 QDs/MWCNTs; that change could boost the stability of LSBs [95].

3.1.4 Quantum Dots in Metal-Air Batteries

Metal-air batteries have the fascinating capability to utilize oxygen from the air at the cathode, converting it into electricity and generating water as a byproduct. This unique process allows for zero pollution. It is worth noting that metal-air batteries possess a remarkably high theoretical energy density, surpassing 1000 Wh/kg [96]. The components of metal-air batteries are metal anode, electrolyte, and air cathode. Typically, iron, magnesium, zinc, aluminum, and lithium make up the anode. [97]. Li-air batteries' extremely high energy density has drawn increased attention in recent years. Higher-performance Li-O_2 batteries require a catalyst that can effectively catalyze both the oxygen evolution reaction (OER) and the oxygen reduction reaction (ORR). Sun et al. have synthesized rich-edge MoS_2 QDs with S vacancy (Vs) employing a unique top-down approach. In a Li-O_2 battery, the innovative Vs-MoS_2 QDs showed impressive ORR/OER activities according to either experimental or theoretical research [98]. To address the issue of over potential, Veeramani et al. synthesized CdSe/ZnS core/shell QDs with CNTs frameworks for a high-performance Li-O_2 battery. The suggested structure provided routes for the transfer of electrons and holes, O_2 gas, and Li ions, respectively. Mn_3O_4 QDs placed on N-doped MWCNTs were developed by Huang and his coauthors as an inexpensive and effective ORR catalyst. After being discharged for 105 h, the composite catalyst showed a specific capacity of 745 mAh/g at 10 mA/cm without voltage loss. It was utilized in a Zn-air battery [99]. Combining $NiFe_2O_4$ QDs with CNTs, which demonstrated a large specific surface area and outstanding conductivity, Xu et al. created a high activity bifunctional ORR/OER electrocatalysts that were employed as the cathode in Zn-air and Mg-air batteries. [100]. CeO_2 QDs on N-doped carbon were prepared by Liu et al., and the Al-air batteries fitted with the resulting catalyst in cathodes demonstrated superior discharge performance [95].

3.1.5 Quantum Dots in Zinc-Ion Batteries

Because of their excellent safety, affordability, and sustainability, rechargeable zinc-ion batteries (ZIBs) have garnered a lot of interest [101]. $ZnMn_2O_4$ (ZMO) QDs in carbon frameworks were reported by Deng et al. as cathode material. Zn/ZMO QD@C batteries' resulting structure significantly improved ZIBs' electrochemical performance [102]. According to Zhang et al., dendritic-free ZIBs are made possible by GQDs because of their improved Zn^{2+} binding. After the 600th cycle at 1 A g^{-1}, the built ZIBs with the vanadium cathode show exceptional performance with a capacity of 164.3 mAh g^{-1} [103].

3.2 QDs in Supercapacitors

SCs are electrochemical energy storage systems that have become of great concern in recent years regarding their outstanding properties including exceptional power density, higher energy density than traditional capacitors, longer cycle life than secondary batteries, good security, and fast charge and discharge abilities [104]. SCs consist of three main components, namely, electrodes, electrolyte, and separator. The most essential component influencing the electrochemical parameters and overall performance of SCs is the electrode material [105]. The practical deployment of SC devices has been struggling due to their poor energy density. According to the energy density (E) equation, SCs' energy density may be improved by increasing either the voltage window (V) or the specific capacitance (C). Furthermore, the kind and mechanism of electrode materials have a considerable influence on the electrochemical characteristics of SCs. Electric double-layer capacitors (EDLC), pseudocapacitors, and hybrid capacitors are the three major types of SCs [106].

3.2.1 QDs in Symmetric Supercapacitors

Electric double-layered capacitors (EDLCs) store charges in a non-faradaic process where the charges are split at the interface between electrode and electrolyte when the electrode is inserted in an electrochemical cell according to the Helmholtz model. The quick reversible insertion/desertion of electrolyte ions onto the electrode surface distinguishes these devices, leading to a high charge/discharge rate and great cyclability. Electrode materials used in EDLCs are mainly carbonaceous material with large surface area which in turn is responsible for enlarging the specific capacitance of EDLC. Nonetheless, because of the low degree of specificity of industrial activated carbon, the energy density of EDLCs is limited, limiting its future practical utility [107]. In EDLCs, the application of QDs and their composites is to embed QDs in carbon electrode materials to boost the specific surface area of carbon materials, resulting in better SC performance.

Quing et al. employed CQDs as nanofillers on graphene fiber in micro-supercapacitors. The interactions inside the composite demonstrated a tremendous boost in mechanical strength and electrical stability via hydrogen bonds between CQDs and the sheets of graphene. The CQDs/graphene fiber assembled device exhibited a high specific capacitance of 607 mF/cm^2, with cycling stability after 10,000 cycles. The inclusion of CD facilitated the rise of active sites and increased capacitance by 22.1%, opening the door for additional ionic channels for conduction [108].

Chang et al. created a 2D porous carbon nanosheet (PCN) made of graphene quantum dots using a facile interface themselves and a subsequent activation technique from a by-product of the commercial sulfonated pitch. It has an extremely thin thickness of 20 nm, a sufficient SSA of 2429 m^2 g^{-1}, a high packing density of 0.562 g cm^{-3}, a high electron-conductivity of 89 S m^{-1}, and a high mechanical elasticity. This PCN may be employed as a powerful EDLC electrode due to its structural benefits [109].

Harper et al. created composite materials by homogeneously coating CDs onto lignin-based activated carbons and decorating the inner surface with CDs binding sites. The obtained CDs have a consistent size distribution range of 2 nm to 5 nm, allowing the crystal lattice to be observed. After the CDs modify the surface of the activated carbons, multiple CDs domains with crystalline structures are evenly distributed on the AC matrix. The composed electrode offers a high capacitance of 301.7 F/g at 0.15 A/g, which is twice larger than that of the AC electrode (125.8F g^{-1}). The specific capacitance of this electrode is 191.8F g^{-1}, with 52% growth achieved with half the number of CDs added, indicating that the increased capacitance is proportional to the quantity of CDs added. These findings suggest that carbon dot modification is an effective technique to increase the performance of ACs, however current research is in its early stages [110].

The surface faradic redox reaction with electrode materials such as metal oxides and chalcogenides is mostly used by pseudocapacitors. Metal oxides QDs and chalcogenides QDs have recently been demonstrated to be viable electrode materials for pseudocapacitor applications. Cong et al. created nonstoichiometric tungsten oxide QDs, $WO_{3\text{-}x}$ ($x = 0.06$), with an average size of 1.6 nm for rapid pseudocapacitor applications. The conversion of the octylamine ligand to pyridine resulted in outstanding conductivity and hydrophilic properties, as well as extraordinary electrochemical activity. In QD electrodes, reversible effective ion transport may be seen during the charging and discharge process. The electrode may profit from a short diffusion path and easy mass/charge transfer due to the QD size and pyridine ligand. Furthermore, 2D layered metal chalcogenides QDs are promising options for capacitive sensors [111].

Yin et al. prepared WS_2 QDs using a simple method and tested their pseudocapacitance features. Surface treatment with 1,2-ethanedithiol improved the characteristics. As a result, a high specific capacitance (457 F g^{-1}) was produced, as well as exceptional cyclic stability over 8000 cycles and Coulombic efficiency (81%) [112].

Elsaid et al. used a simple hydrothermal technique to prepare $FeCo_2S_4$ rod-like structures on nickel foam. The $FeCo_2S_4$ nanorods electrochemically demonstrated a high specific capacitance of 4035 F g^{-1} at 1 A g^{-1} as a current density in 6 M KOH solution, with outstanding cycling stability of 80.4% capacitance retention and 100% coulombic efficiency after 5000 cycles. The symmetric device has the highest specific energy (37.72 W h kg^{-1}) and specific power (603.6 W kg^{-1}) at a current density of 0.5 A/g, as well as extraordinary cycling stability of 80.3% capacitance retention after 5000 cycles and high coulombic efficiency of 94% [113].

3.2.2 QDs in Asymmetric Supercapacitors

Synthesizing anode materials with high specific capacitance remains a big difficulty for building asymmetric supercapacitors (ASCs) with high energy density. Among the many approaches, producing ultrafine QDs distributed in a carbon substrate and creating ASCs can be a useful way to address the current problems. Zhihui et al. created a C-$NiCo_2O_4$/C@Ni-Co-S electrode by connecting the core of 0D/2D carbon-modified $NiCo_2O_4$ and the exterior sheath of Ni-Co-S nanosheets. The 0D carbon quantum dot doping efficiently avoids agglomeration of $NiCo_2O_4$ nanoparticles while improving electric conductivity. The use of 2D carbon nanosheets as an intermediate layer can increase the durability of the electrode. Ni-Co-S nanosheets can enhance the electrode's area-specific capacitance and hence the energy density of the device. The formed electrode offers a remarkable specific capacitance of 2396 F g^{-1} (7.9 F cm^{-2}) at 3 A/g and good cycle stability (92% after 10,000 cycles). A semi solid asymmetric supercapacitor (ASC) employing this composite as a positive electrode against activated carbon (AC) as the negative electrode offers a high energy density of 95 Wh kg^{-1} at 0.9 kW kg^{-1} and an remarkable cycle performance [114].

Zhang et al. created a precursor using a zeolitic imidazolate framework (ZIF-8) and carbon nanotubes (CNTs). As electrodes for ASC, both ZnO QDs/C/CNTs and N-doped carbon/CNTs were used. The device achieved a working potential of 1.7 V while providing high energy density (23.6 Wh kg^{-1}) and power density (16.9 kW kg^{-1}) [115].

Fe_2O_3 is also a good anode material for ASCs. However, the Fe2O3-based anodes continue to have a poor specific capacitance. Using a simple method, Xia et al. created a hybrid material containing Fe_2O_3 QDs evenly placed onto functionalized graphene nanosheets (FGS). In 1 M Na2SO4, the hybrid material displayed a significant specific capacitance of 347 F g^{-1} between -1 and 0 V vs Ag/AgCl. A high-performance ASC operating at 2 V was built using Fe_2O_3 QDs/FGS as the anode and MnO_2/FGS as the cathode. At 100 W kg^{-1}, the supercapacitor had a high energy density of 50.7 Wh kg^{-1}, as well as strong cycle stability and power capabilities [116].

Jinfeng et al. created a composite of Ni_3S_4 (QDs)@(G/CNTs). The addition of G/CNTs to Ni_3S_4 QDs has increased electron interaction, promoting electron transport in the material. G/CNTs enhanced the specific capacitance of Ni_3S_4 QDs by 4.5 times and boosted their rate capability from 22 to 82%. The energy density of an

asymmetric supercapacitor built with Ni_3S_4 QDs@G/CNTs and activated carbon electrodes was 50 Wh kg^{-1} [117].

Gamma radiation was employed by Abdel Maksoud et al. to aid in the manufacture of Ag adorned nanoplates spinel-type oxide NiMn2O4 as a potential electrode material. The Ag@ $NiMn_2O_4$ electrode has a capacitance of 622 Fg^{-1} at 1 Ag^{-1} current density while preserving outstanding cycle stability (capacitance retention of about 88.8% and coulombic efficiency of 97.6% at 10 Ag^{-1} after 5000 cycles). An asymmetric supercapacitor (Ag@ $NiMn_2O_4$ //rGO) with a maximum specific density of 30.44 Wh kg^{-1} and a specific power of 10.3006 kW kg^{-1} within a broad potential window of 1.6 V has been built. Furthermore, the capacitance of the Ag@ $NiMn_2O_4$// rGO hybrid supercapacitor device is around 94.8% retained and exhibits almost 100% coulombic efficiency [118].

Some recent research has employed quantum dots directly as electrodes, which have substantial benefits as electrode materials for micro-supercapacitors (MSCs) [109]. Molybdenum disulfide quantum dots (MoS_2-QDs) and nitrogen-doped GQDs (N-GQDs) were utilized as cathode and anode materials, respectively, by Liu et al., and the resulting (MSCs) showed outstanding electrochemical performance [119].

3.2.3 QDs in Hybrid Supercapacitors

Both faradaic and non-faradaic processes are exploited for benefit in hybrid supercapacitors. Manufacturing hybrid SCs with the purpose of merging the features of high energy in batteries and high power in SCs is particularly encouraging. Hybrid supercapacitors generally employ a capacitor-type positive electrode and a battery-type negative electrode [120]. Diab et al. used a simple solvent evaporation technique followed by a hydrothermal reaction for supercapacitors to create a hybrid structured GQDs/CuO. CuO aggregates are created by employing a Cu-MOF complex as the structural and compositional predecessor, and GQDs as an external support. The combined GQDs/CuO electrode provides a high specific capacitance of 729 F g^{-1} at a current density of 1 A/g and good-rate capability, as well as increased cyclic performance (82.2% retention after 3000 cycles) as a supercapacitor cathode material. The as-fabricated ASC has an energy density of 32.2 W h kg^{-1} at a power density of 748.9 W/kg and an enhanced cyclability of over 8000 cycles, thanks to the enriched electrochemical active sites and intimated connection between Cu species and doped GQDs [121].

Elsaid et al. prepared a new nano coral-reef $ZnFeNiCo_2S_4$ quaternary sulfide on a Ni Foam (NF) substrate in two simple hydrothermal phases for enhanced battery-type SCs. On the Ni foam, the $ZnFeNiCo_2S_4$/NF electrode was effectively produced as a binder-free electrode. At 6 M KOH as the electrolyte, the electrochemical analysis of the $ZnFeNiCo_2S_4$/NF revealed a high specific capacity of 1778 C g^{-1} at a current density of 3 A g^{-1} with outstanding cycle stability of 86.1% capacity retention and 100% coulombic efficiency. The specific capacitance of the battery-type symmetric supercapacitor was 162.3 C g^{-1} at a current density of 2 A g^{-1} with a specific energy of 33.8 Wh kg^{-1} and a specific power of 2519.25 W kg^{-1} [122].

Xiang et al. developed a ternary composite of (GO/CQDs/Polypyrrole) that may be employed as an electrode-active material in a supercapacitor. At a current density of 0.5 A g^{-1}, GO/CDs/PPy has a specific capacitance of 576 F g^{-1}, and a supercapacitor made of two symmetric GO/CDs/PPy electrodes has a high energy density of 30.1 Wh kg^{-1} at a power density of 250 W kg^{-1}. More notably, after 5000 cycles, the as-fabricated supercapacitor maintains remarkable cycle stability [123].

3.3 QDs for Hydrogen Storage

Photocatalytic hydrogen evolution is an environmentally friendly technique of storing energy. QDs are significant because of the quantum confinement effect, which allows them to create numerous excitons, enhancing charge separation and transmission, and boosting photocatalytic activity [124]. There are three types of hydrogen storage technologies: physical, chemical, and hybrid. Now, compressed hydrogen storage is the most established and widely accessible technique. Graphene QDs have a high specific surface area and can be readily doped with transition and alkali metals, which increases hydrogen storage capacity significantly. Few research attempts to dope Pb [125], Cu [126], and other metal QDs to increase hydrogen storage performance. No QDs composite materials for hydrogen storage have been developed.

4 Challenges and Future Motivation

QDs may play an important role in the design of microsupercapacitors or microbatteries in the future. The extra functional groups on the surface of QDs can be exploited to manipulate their shape. The improved chemical stability and photostability of various types of QDs can be investigated further to find a role in future photochargeable supercapacitors or batteries. Because of their synthesis techniques, the large-scale synthesis of QDs remains a source of worry. This might stymie the commercialization of QDs. Researchers are looking at natural precursors as a source for environmentally benign, low-cost, and non-toxic QDs. This might pave the way for a wide range of studies employing cleaner technology.

5 Conclusion

In summary, this chapter provides an overview of quantum dot composites' substantial capabilities in the development of high-performance energy storage devices. With the advancement of improved technologies and characterization methodologies, interesting new research fields based on QDs may emerge.

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Life Cycle Assessment of Quantum Dots and Its Composites

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Abstract In this chapter, we take a close look at the environmental friendliness of quantum dots (QDs) and their composites via the lens of a life cycle assessment (LCA). The importance of QDs and their widespread practicality are first elucidated. The environmental effects of extracting raw materials, producing, transporting, using, and disposing of QDs are all considered in the LCA analysis. This chapter focuses on the trade-offs between performance and sustainability, and it explores the environmental effects of various QD composite materials. QDs' use in low-power uses like lighting and screens is also highlighted. Carbon emissions, resource use, and trash output are only some of the environmental measures used to calculate the

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extent of these effects. This chapter provides a thorough analysis of the environmental impacts of quantum dots (QDs) in an effort to educate researchers, industry professionals, and policymakers so that they can make educated decisions about the research, application, and management of QDs and their composites.

1 Introduction

The environmental impact of composites is measured across their full life cycle using a method called life cycle assessment (LCA). It's an all-encompassing method that considers the composite material's lifecycle from raw material extraction to final disposal [1]. The fundamental objective of a life cycle assessment (LCA) is to provide an all-encompassing view of the environmental effects connected with composites by quantifying and assessing the energy consumption, emissions, resource utilisation, and waste generation at each stage. Composite materials' life cycle kicks off with raw material extraction and processing. Carbon fibres, glass fibres, and different resins are common components of composites [2]. The mining, refining, and processing of these basic materials all have environmental implications that may be assessed using an LCA at this point. It takes into account things like energy use, water use, and pollution caused by these procedures. Composite material fabrication and manufacturing is the next step. Manufacturing processes in this sector range from pultrusion and filament winding to resin transfer moulding. The LCA analyses the resources used, pollution released, and trash produced by these activities [3]. Energy is expended to shape and bond materials and emissions are released into the atmosphere [4]. If you want to know how alternative manufacturing methods could affect sustainability, you need to know the environmental impact of the production phase [5]. Distribution and transport are also crucial components of a life cycle assessment. At this point, we factor in the emissions and energy used to move raw materials to production facilities and completed composite products to consumers [6–8]. The environmental impact of composites can be greatly affected by factors like as the mode of transportation utilised and the geographic distance between suppliers, manufacturers, and consumers. After composites have been put into practise, their effect on the natural world is still being studied. Composite parts are commonly used in the aerospace sector, for instance [9]. The environmental benefits of employing composites instead of traditional materials can be determined by evaluating aspects such as fuel economy, energy savings, and emissions reduction during the use phase. The life cycle of composites also includes the crucial step of maintenance and repairs [10]. Consequences to the environment, such as the energy and materials needed for repairs or replacements, should be taken into account when dealing with composites. The long-term durability of composites can be seriously compromised by these actions [11, 12]. Composites' end-of-life management is the last phase of their LCA. In this stage, we examine the processes used to dispose of or recycle composite materials once they have served their purpose. It considers the ecological effects of various

waste management options such landfilling, incineration, and recycling. To lessen the impact that composites have on the environment, proper end-of-life management is crucial.

2 Background of LCA of Materials

Rising global consumption of plastics over the past few years has resulted in a rise in plastic trash. About half of all plastics are put to use in packaging and agricultural films are two examples of single-use usage [13]. Pipes, cable coatings, and structural materials only account for 20–25% of all plastic usage. The rest goes into applications with a shorter to medium-term lifespan in the consumer market, such as electronics, furniture, and auto parts [14]. The short useful lives of plastic products contribute significantly to the difficulties inherent in plastics disposal. Plastic packaging is just one example of an area where this period can be significantly less than a month.

Because of their low density and widespread use in hollow objects (making their apparent density even lower), plastics are a glaring problem in our landfills and oceans [15]. However, while plastics may account for 20–30% of the volume weight fraction of MSW, they account for just 7–9% of the overall MSW mass [16]. However, plastic trash can be found in much larger concentrations in certain streams, such as those from the manufacturing and service sectors. The fact that plastics are typically not biodegradable means they will stay in the environment for a very long time and contribute to the problem [17]. The public's awareness of the plastics problem has grown as a result of their pervasiveness. In response, numerous LCAs have been conducted to analyse the effects of plastic items over their entire lifespan because of public interest in the topic. Recently, economic evaluations have been added to the mix to round out the research.

3 LCA of Polymer Composites

Cradle-to-factory gate and cradle-to-grave are the two most popular systems utilised in LCA research. All processes from raw material and fuel extraction to final product delivery at the factory gate are factored into a cradle-to-factory-gate LCA analysis [18]. Material manufacturers frequently provide Cradle to Factory Gate studies. Every stage, from initial creation to final disposal, is accounted for in the cradle-to-grave system. The full life cycle can be accounted for in cradle-to-grave studies. Since waste management varies from nation to country and not all waste treatment alternatives may be considered, the results of cradle-to-grave evaluations for the same product can vary greatly [19]. Cradle-to-factory-gate assessments can provide initial insight into environmental implications if comparisons across different waste disposal strategies are unavailable. Multiple formats exist for reporting LCA findings. Results from the "middle" of the range are typically given in studies. At this

stage, information from the life cycle inventory is transformed into environmental effect categories, such as the contribution to global warming or acidification. The final environmental score for a product is determined by adding up the results from several impact categories using appropriate weighting factors. Single-score analyses are another name for analyses with a focus on the final score. The weighting factors for the different effect categories are determined based on subjective assessment. When one product is demonstrably superior to another in all impact categories (or is comparable to the other option in all categories), it may be possible to draw conclusions from a comparative LCA research based on results from the middle of the range. If, on the other hand, some types of environmental consequences are more severe than others, then prioritisation decisions must be made among the many types of impacts.

A natural target of the movement to get rid of everything that isn't "green" appears to be plastics [20]. The huge amount of energy they contain and their pervasiveness as litter are typically highlighted when discussing their impact on the environment. Recent quantitative investigations, however, rarely back up this prejudice [21]. In fact, plastics typically show very favourable life cycle (LC) profiles when compared to other materials in studies evaluating the environmental and economic effect of alternative materials. In terms of Global Warming Potential (GWP) and Total Energy Use (TEU) [22]. The current significance of greenhouse gases enhancement led to the selection of these classes of environmental impact. Additional areas of environmental impact are reported by some research. These include the possibility for ozone depletion, photochemical oxidation, acidification, and eutrophication. The findings reveal that, contrary to popular belief, ordinary polymers typically cause fewer adverse effects on the environment in terms of GWP and TEU than do other materials. Reuse, which prevents the use of nonrenewable resources, has also shown to have a positive effect on both measures of environmental sustainability.

Many engineering projects hinge on the material choice made, since it affects the longevity, cost, and manufacturability of the end result. In addition, manufacturers are under increased regulatory pressure from government agencies to reduce the environmental impact of their operations and the goods they produce. Since recycling potential and/or end-of-life disposal options differ from material to material, material selection can be particularly important for green design. Identifying multiple mechanical, electrical, chemical, thermal qualities, environmental impact variables, and life cycle costs of candidate materials is the first step in modern integrated design processes (IDP) for systematically selecting the optimal material for a specific application (Fig. 1). For an IDP to be successful, multidisciplinary design teams must collaborate from the start of a project to provide solutions with many uses [23]. However, when several criteria from several fields must be met in a material selection problem, complications arise due to potential conflicts between the criteria and/or the relative relevance of the various criteria. Additionally, correct indices within each area require specialist expertise to define [24]. For a wing spar's leaf spring/beam, for instance, it may be preferable that it not only be light but also strong enough to withstand a specified bending force without buckling.

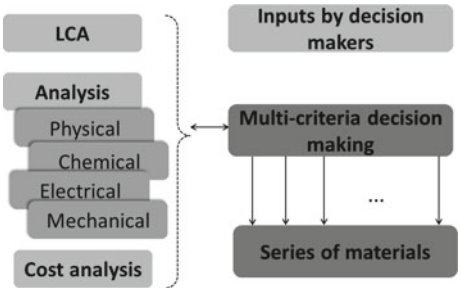

Fig. 1 A diagram showing how to choose materials in an integrated design process

4 LCA of Quantum Dots and Its Composites

Specifically designed and manufactured materials having dimensions between 1 and 100 nm are known as engineered nanomaterials (ENMs) [25]. In addition, ENMs show unique characteristics in comparison to bulk materials of the same composition [26]. The environmental implications of ENM production and use are unknown, despite the fact that their commercial production volumes have increased [27]. Therefore, in order to guarantee their sustainable production and utilisation, it is crucial to have a thorough awareness of the impacts made by ENMs throughout their life cycle. Since LCA attempts to quantify the environmental consequences of a system over the course of its life cycle [28], it is the most suited instrument for tackling this challenge [29]. However, the environmental evaluation of a significant and unique ENM, carbon dots (CDs), has not been the subject of any prior research. Carbon nanospheres are a new type of nanoparticle with a round shape and a size between 1 and 10 nm [30]. Nonetheless, making them in a way that doesn't harm the environment hasn't received much attention. Consequently, it is important and essential to provide information on the potential environmental implications of representative synthesis processes for the creation of CDs [31]. Here, we apply a cradle-to-gate life cycle assessment (LCA) to compare and comprehend the environmental effects of carbon dots (CDs) produced using six distinct bottom-up synthetic techniques. The most common methods now used to synthesise CDs are hydrothermal synthesis and microwave-assisted synthesis, both of which use citric acid as a starting material (and sometimes urea as well). According to the findings, power consumption is the most significant environmental factor in hydrothermal synthesis, while citric acid is the main source of pollution in microwave-assisted synthesis. Rescaling results using the CDs' fluorescence quantum yield allowed for a performance-based comparison as well. This method substantially altered the previous preferred order across all classes. The most environmentally friendly method, according to a recent study, was the microwave-assisted synthesis of citric acid-derived CDs; however, today both urea- and citric acid-derived CDs can be synthesised (by hydrothermal or microwave-assisted treatment) [32]. Different acid catalysts based on transition metal phosphates, such as vanadium and niobium, have been evaluated for their performance in the hydrothermal synthesis of carbon dots (CDs). Commercial xylose and

liquor of xylose extracted from olive pits were used as the carbohydrate sources. The NH3-TPD, DTA/TG, XRD, and XPS methods were used to determine the identity of the catalysts. At a temperature of 180 degrees Celsius, the reaction was run for 4 h. Analysis of the properties and characteristics of CDs nanoparticles revealed their existence, regardless of the carbohydrate source. Simultaneously, highly fluorescent N-doped CDs were synthesised via the same hydrothermal method, and their photocatalytic activity was studied. To compare the environmental consequences of the synthesis using commercial xylose to those of the synthesis using biomass, a Life Cycle Assessment (LCA) was performed on both synthesis methods [33]. Organic light-emitting diodes (OLEDs) and inorganic nanoparticle-based quantum LEDs (QLEDs) are two rival forms of light-emitting technology used in displays. For these purposes, innovative nanomaterials and organics can be found in the next substance classes of perovskites and Q-OLED displays. However, due to their complexity, intrinsically diverse structures, and rapid growth in the literature, assessing the safety and viability of these emissive compounds in a timely and comprehensive manner is challenging. To compare these potential alternatives to incombent cadmium-containing quantum dots, we propose adopting an alternatives assessment centred on danger, cost, and performance. Chemical substitution is a growing trend, and this type of assessment is employed by both industry and government. It makes advantage of existing data while highlighting crucial information gaps that must be considered. The low quantities required in their application for display make OLEDs cost-effective, although performance evaluations do not single out a superior option. According to the risk assessment, there is no better option because each unique nanomaterial or organic compound has its own set of drawbacks [34]. The study provides a framework for future researchers to analyse their own unique drugs, and the results highlight the need for a low-hazard high-performance replacement substance. New domains of application can be explored with the advent of emerging photovoltaic systems (EPVs) such organic solar cells, dye-sensitized solar cells, perovskite solar cells, and quantum dots solar cells. Life cycle analysis is essential for determining the environmental impact of EPV technology developments and sophisticated materials. Materials and production methods responsible for the bulk of a product's environmental effect can be isolated with the help of life cycle evaluations (LCAs). Recent life cycle assessments (LCAs) have shown that EPV production may result in reduced energy consumption and faster energy payback time compared to conventional PV technologies, although these results are sometimes hard to compare due to differences in methodology and system boundaries. However, the examined LCAs also identify some "environmental hotspots" regarding the materials, energy, and chemicals used. The existing use of vital raw materials, precious metals, and hazardous as well as energy-intensive products means there is still opportunity for optimisation in terms of environmental sustainability and the circular economy [35]. Carbohydrates, lipids, phenolic compounds, and proteins are only few of the many organic substances found in spent coffee grounds (SCGs). As a result, we looked at them as a feasible option for obtaining carbon dots (CDs) via a nanotechnology method. In this study, CDs made by SCGs were compared to those made from more conventional precursors like citric acid and urea. The SCG-based CDs were made

by carbonising solid samples in a single pot without using any solvents, yielding particles on the nanoscale (2.1–3.9 nm). These nanoparticles displayed the signature blue fluorescence of carbon dots as well as modest quantum yields (2.9–5.8%) and excitation-dependent emission. The SCG-based CDs showed promise as fluorescent probes for Fe3+ in water that are also environmentally relevant. Furthermore, life cycle assessment studies confirmed that making CDs out of SCG samples is a more environmentally friendly way than making CDs out of classic reported precursors, when looking at it from both a weight-based and a function-based perspective [36]. Among the many potential uses for quantum dot nanoparticles (NPs) is in photon upconversion devices, which boost the solar panels' ability to convert light into power. In this study, we present ready-to-use LCA unit process data for four NPs (cadmium selenide, cadmium sulphide, lead selenide, and lead sulphide) that are well-suited for photon upconversion applications. The information is presented for two potential futures: an optimistic and a pessimistic one. The effectiveness of the NPs in mitigating climate change is evaluated using an impact assessment, which reveals that solvent-related operations, like steam production for recycling and hazardous waste treatment, are major contributors to this issue. To demonstrate the relevance of the findings, an upconversion-layer solar module is evaluated prospectively to determine whether adding more solar modules or retrofitting existing ones with upconversion devices is preferable from a climate perspective [37]. The evaluation reveals that, depending on the circumstance, solar modules would need to increase their efficiency by 0.05 to 2 percentage points per gramme of applied NPs for the upconversion layer to be desirable.

5 Conclusions

The environmental implications of well-established processes and products can be effectively evaluated with the help of life cycle assessment (LCA). However, incorporating it into decision-making for the long-term success of cutting-edge technology presents a formidable obstacle. It is challenging to do LCA assessments at the outset of product design for new nano-enabled products (NEPs) due to the high levels of uncertainty and lack of data along the whole value chain. Data scarcity and quality difficulties are common for LCA practitioners working on developing technologies due to the proprietary nature of industrial data, necessitating some assumptions based on prior scientific literature and industry reports. These assumptions are standard practise in LCA, and while they are not false, they are likely to add some degree of uncertainty to the findings. To facilitate the growth of collaborative research across the life cycle and the generation of fresh datasets, the dLCA framework has been established. By feeding the experimental data back into the LCA model, uncertainty in various LCA stages can be reduced with each iteration, while also pointing experimentalists in the direction of questions they should be asking in the future. Although this research focuses on quantum dot (QD) applications,

the suggested dLCA framework and the interdisciplinary cooperation it encourages can be applied to the estimation of environmental impacts across a wide range of developing technologies.

Future developments in assessment methodologies for Life Cycle Assessment (LCA) of quantum dots (QDs) and their composites will take into account shifting synthesis methods, expanding applications, shifting regulations on nanomaterials, and a heightened emphasis on circular economy principles and recycling practises. In addition to promoting better data gathering, transparency, and accessibility, future LCA studies will cover a wider range of uses across a variety of sectors. The results of life cycle assessments (LCAs) will also be used in real-time decision making to promote ethically sound product and policy creation. Researchers hope that by effectively disseminating their findings, LCA studies will help raise public awareness of the environmental impact of QDs and ultimately lead to policy changes that promote sustainability and environmental responsibility in the QD industry.

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Theory, Modeling and Simulation of Quantum Dots and the Multiphase Systems

Siddiqui Fatima Sarah and Sunita Bhosle

Abstract To understand the complex behaviour of quantum dots in multiphase systems, this chapter of a book delves into the theoretical foundations, advanced modelling approaches, and sophisticated simulations used to accomplish so. Nano-sized structures known as quantum dots show great promise for a variety of uses due to their exceptional electrical and optical characteristics. To fully appreciate and make use of these attributes, a thorough theoretical foundation is required. The confinement effects and energy levels that determine the behaviour of quantum dots are first explored in this chapter, which delves into the fundamental quantum mechanical principles regulating quantum dots. Next, it delves into cutting-edge modelling techniques, such Density Functional Theory, for making precise predictions about quantum dot properties. The effect of quantum dots' interactions with various materials on optical, electrical, and thermal properties is discussed, and extended to multiphase systems. This chapter utilises state-of-the-art simulations to provide light on the design and optimisation of devices based on quantum dots, with a particular emphasis on fine-tuning their features for use in next-generation photonics, electronics, and renewable energy technologies.

Keywords Quantum dots · Theoretical foundation · Modelling · Photonics · Electronics

1 Introduction

Quantum dots (QDs) are nanometer-sized systems that exhibit quantum confinement in all three spatial dimensions due to their small size [1–5]. Typically smaller than 20 nm in size, colloidal QDs are fluorescent semiconducting nanocrystals having a radius comparable to the Bohr exciton radius of the material [6]. Colloid chemistry has advanced greatly in recent decades, allowing for the manufacture of high-quality

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semiconductor nanocrystal QDs. Compared to organic dyes and polymers, quantum dots have improved chemical and photo-stability as well as a high photoluminescence quantum yield and a broad absorption spectrum that overlaps with the solar spectrum [7, 8]. In highly sensitive non-isotopic detection methods, colloidal QDs have been frequently used as biological tags. It is a viable contender for use in light-emitting diodes, nonlinear optics, laser media, and photovoltaics due to its adjustable emission, strong photo-oxidation stability, and electron conductivity [9].

The energy and spacing of discrete electron levels are strongly dependent on crystal size in nanocrystals, making them accessible to scientific observation and practical use. Since quantum dots have electron states on a higher scale than the lattice constant of a crystal, they are sometimes referred to as mesoscopic atoms or artificial atoms. A quantum dot (QD) can be any size up to and including a macromolecule or a single impurity atom in a crystal. Changes in transport and optical properties are profound when electron energy are quantized in nanometer-sized crystals. The dependency of the fluorescence wavelength on the size and material composition of the nanocrystals is illustrated in Fig. 1. Using materials with varying band gaps (CdSe for blue, InP for green, and InAs for red) leads to significant changes in emission wavelength. Within each colour, the size of the QDs determines the degree of fluorescence emission.

Discrete energies occur when the size of the system is on par with the Bohr exciton radius at the nanoscale, which results in a high effective band gap and a blue shift in the optical transition. Enclosing the QD within an organic surfactant is a common method for achieving quantum confinement in QDs. Therefore, the typical QD has an inorganic core surrounded by organic surfactant ligands. Type II–VI, IV–VI, and III–V semiconductors, including (CdS) ZnS, (CdSe) ZnS, (CdSe) CdS, and (InAs) CdSe, are usually used to construct core–shell quantum dot (QD) systems [10]. The perovskite solar cell has emerged as a significant member of the core–shell QDs family in recent years [11]. The most promising optical gain medium for the advancement of solar cells was thought to be the organic–inorganic halide perovskite material ($CH_3NH_3PbX_3$, X = Cl, Br, I), due to its huge optical absorption coefficient and high carrier mobility [12].

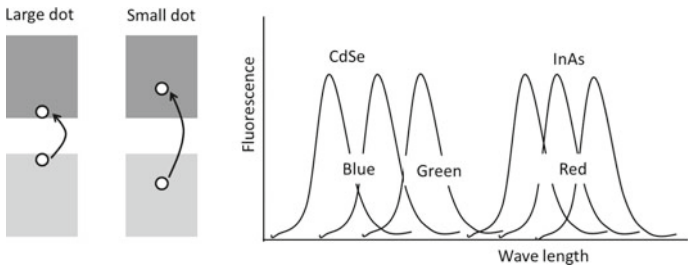

Fig. 1 The band gaps of the materials cause significant variations in the fluorescence wavelength. The varying diameters of the NCQDs define the wavelength for each of the three primary colours

The study of QDs primarily makes use of two methods: experiment and computational simulation. The optical, electrical, and thermal properties of QDs, as well as their sensitivity to changes in size, shape, and surface passivation, have been the subject of extensive experimental and theoretical study. The eventual design and production of colloidal QDs relies heavily on experimentation, however this approach presents significant obstacles due to its high cost and lengthy development cycle. In order to keep the optical, electrical, and thermal properties of typical QDs intact despite their susceptibility to environmental changes, special attention must be taken in their design. Extreme difficulty is posed by the fact that experimental examinations of QDs' surface configurations may be costly and time-consuming. Core–shell QDs frequently use chemical functionalization, although it is challenging to realise surface decorations with specified functional groups. QDs' electrical states, on the other hand, are crucial to comprehending their characteristics. Common experimental methods, however, do not yield usable electronic images at the atomic scale. Because of the nanoscale nature of QDs, computational simulation has emerged as a crucial tool in our quest to learn more about them. Numerical simulations from basic principles and classical molecular dynamics (MD) have been employed extensively in difficult issues for a long time. Modern solutions to the issues encountered in experimental research can be found in computational approaches. They can consider the bulk surface and defects on an equal footing and add electronic correlation effects using the first principles method, which yields an accurate atomistic description of the electronic structure.33 Using the density functional theory (DFT) technique, researchers have proposed a new device based on graphene QD for DNA sequencing, which should serve as useful guidance for future experiments [13].

2 Quantum Dot Simulations and Common Computational Methods

Quantum dots (QDs) have had their electrical and optical properties, which are regulated by the density of states (DOS), studied extensively through first principles computational technique [14]. The ab initio method, or first principles method, is a technique for numerically solving the Schrodinger problem. In order to numerically solve the Schrodinger equation, or more accurately the Kohn–Sham equation, the DFT method is commonly employed [15]. Traditional self-consistent first principles local-density approximation (LDA) calculation is limited to calculations involving a few dozen atoms at most, and is written as [16];

$$E_{xc}^{LDA} = \int \rho(r)\varepsilon_{xc}(\rho)dr,$$

where ρ is the per-particle exchange correlation energy in a homogeneous electron plasma of density.

Calculating the near-band-gap single electron states relevant to the optical and transport properties of thousand-atom colloidal QDs has been proposed as a much faster alternative to LDA that scales linearly with system size, mitigating the extensive computational effect brought by traditional self-consistent first principles method [17].

It has been noted that the energy gap may be underestimated by LDA and generalised gradient approximation (GGA) methods when calculating principal energy [18].

So, the modified Becke Johnson (BJ) and the Engel-Vosko generalised gradient approximation (EVGGA) formalism48 were put forward. It was stated that the exchange correlation energy and its charge derivative could not be accurately reproduced using LDA or GGA. The exchange energy can be reproduced with less precision using EVGGA and BJ, but they are still capable of replicating the exchange potential [19].

It is possible to determine the QD potential by;

$$P_{QD} = \frac{b}{a} E_g \times 10^{-3} \lambda,$$

Parameter for semiconductors, where E_g is the energy gap and b/a is a constant. The indirect energy gap can be computed using DFT by employing the full potential-linearized augmented plane wave approach. Within the non-overlapping spheres, the wave function and charge density are extended using spherical harmonic functions, while outside, the wave function and charge density are expanded using a basis set of plane waves. The exchange correlation potential within a GGA framework can be used for total energy estimates.

It provides the maximum thermal conductivity of a material and can accurately represent ballistic thermal transfer. Thermal transport can be thought of as ballistic and characterised by the Landauer formula when the system size of a nanomaterial is smaller than its phonon mean free path [20].

$$I = \int_0^\infty \frac{d\omega}{2\pi} \mathrm{h}\omega\tau[\omega](f_L - f_R),$$

Here $\tau[\omega]$ is the transmission coefficient.

3 Optical Properties by Computational Simulations

Colloidal semiconducting QDs are promising emitters for luminescent solar concentrators with potential uses in high-efficiency and cost-effective photovoltaics due to their broad absorption spectra spanning the ultraviolet to the near infrared. QDs can have their absorption and emission spectra tuned to minimise energy loss due

to reabsorption. Because QD's quantum confinement is size, shape, and chemical composition dependant; its optical properties can be precisely tuned through design. Despite intensive work on the core–shell QD systems' fabrication [21], The development of well-behaved colloidal QDs may be hampered by the lack of in-depth research into the optical properties of these core–shell QDs, which has thus far hampered progress in these areas. In order to improve photocatalytic capabilities, it is essential to absorb solar energy and undergo photo-induced charge separation and transfer. Photon losses at subgap energies and the decay of electron–hole pairs limit the highest efficiency of conventional solar cells to the Shockley-Queisser (SQ) limit (33%). Using intermediate band (IB) as a subgap photons absorber is a viable approach to breaking the SQ limit [22]. The optical performance of QD structures was intensively worked on using computational methods. In order to apply the IB idea to solar cells, colloidal QDs have recently been proposed as a platform. The IB states may be clearly distinguished from the margins of the valence and conduction bands in arrays of surface-reconstructed CdSe dots, as shown by Vörös et al. using a first-principles approach. Cobaltocene-doped CdSe QDs have their electron affinity and ionisation potential measured by their total energy difference [23]. When the electron affinity for the cobaltocene is greater than the ionisation potential of the CdSe, electron transfer occurs. Cubic binding energy of 0.16 eV/QD for CdSe QDs indicates stability in the absence of ligands at ambient temperature.

In addition to the aforementioned core–shell QD systems, there is a plethora of carbon nanodots based QDs that vary greatly in size, edge topology, and functional groups. After simulating 2D graphene QDs with high-resolution transmission electron microscopy and performing tight-binding computations of their optical spectra using a time-dependent density functional, Ghosh et al. suggested multiple atomistic models of these materials. Light absorption at the visible range has been decoded by locating the dominant electron and hole orbitals [24]. Armchair edges contribute heavily to the DOS and result in a clear band gap in the graphene QDs model at the nanoscale length scale. It is also determined that the introduction of a controlled number of hydroxyl (OH) or carbonyl (COOH) groups into graphene quantum dots is an efficient way to fine-tune their excitation energies in the visible spectrum.

4 Electronic Properties by Computational Simulations

QDs have a highly variable electrical structure that is mostly determined by their size and surface conditions. Traditional QDs, consisting of a semiconductor or metal core or shell, may find usage in electronics. Although polymeric materials are simple to manipulate, they also have a low dielectric field. Ceramic materials, on the other hand, have a greater dielectric constant but are more difficult to synthesise. Engineers can more easily access the polymers' inside through a core–shell structure that combines it with a ceramic. The high capacitance of these materials makes them useful in a variety of electrical contexts.

By coating the core particle in a shell, we can boost its colloidal stability and shield it from photodegradation. Many inorganic components, including silicon and silica, are employed in core–shell QDs. Using a first-principles approach, Reeves et al. investigated the effect of surface termination on phonon-mediated relaxation of an excited electron in a silicon-based QD structure [25]. Surface passivation for silicon QD with fluorine atoms was found to significantly slow down hot electron relaxation, it was stated. The relaxation period is mostly determined by the rate at which the heated electron is shuttled between energetically neighbouring states, and specific electronic states are able to hold the electron for longer than others.

The behaviour of the electrons during relaxation in QDs can be characterised by [26]:

$$\langle E(t)\rangle = \sum_i \varepsilon_i(t) p_i(t),$$

where E(t) is the ensemble-averaged energy, ε_i is the electronic state *i* and $p_i(t)$ is the state probability.

The impact of silicon QD size on hot electron relaxation was studied by Wong et al.86. Large QD have a lesser surface area to volume ratio than small QD, hence the effect of fluorine passivation is mitigated [27]. Decoherence and the impact of quantum dot size on this result connected with fluorine passivation were also explored. The relaxation time constant decreases dramatically, and the characteristic hot electron shuttling behaviour disappears, as the size of the silicon quantum dot increases. However, decoherence can halt the hot electron relaxation by a factor of two or more. Our research shows that the quantum Zeno effect, in addition to the surface-specific vibronic effect for tiny quantum dots, can cause gradual hot electron relaxation.

Core–shell QDs can be split in half based on their electrical states. If the minimum of the conduction band and the maximum of the valence band are located in the same material, we say that the core–shell structure is of type-I. They are considered type-II if they are notable changes in their electrical structures. The core–shell CdSe/CdS and CdSe/CdTe QDs electronic states were studied by Li et al. using a first-principles method. CdSe/CdS and CdSe/CdTe QDs are proven to be type-I and type-II heterostructures, respectively, based on their inherent band-offset alignments [28]. CdSe/CdTe QDs have an extremely distinct hole state from CdS/CdS QDs, with the hole wave function being localised within the shell in the former and the core in the latter.

Size, shape, and edge morphology all play important roles in determining the energy gap in 2D monolayer based QDs such silicene, graphene, and hexagonal boron nitride (h-BN), in addition to the core–shell structure [29]. The influence of edge passivation with OH and CN groups and H, F, O, and S elements on the electrical characteristics of hexagonal and triangular shaped silicene QDs with armchair and zigzag borders is studied using first principles DFT approach [30]. Edge passivation by various atomic and molecular families is studied for its effect on the stability and electrical characteristics of hexagonal and triangular silicene

quantum dots. Depending on the pieces or groups that are attached, the structures undergo significant shape changes. Sulfur-passivated zigzag triangular flakes and flakes with OH groups linked to their edge atoms exhibit the most striking morphological changes. A significant total dipole moment is manifested in a spherical form. Positive binding energies have been calculated for all the clusters that have been investigated, proving their stability. In zigzag hexagonal-H (zigzag triangular-H) and zigzag hexagonal-S (zigzag triangular-OH), a flexible structural transformation from insulator (conductor) to conductor (insulator) is obtained. Flakes with an even number of Si atoms will be antiferromagnetic, while flakes with an odd number of Si atoms can be either ferromagnetic or antiferromagnetic, depending on the attached element or group, due to the parity of the total number of Si atoms. As a result, silicene flakes can have their characteristics modified for specific uses by selecting the appropriate functional groups or components to attach to them. Clusters passivated with S or OH are insensitive to edge defects and have tunable electronic properties, making them promising in semiconductor device applications; in contrast, hydrogenated or fluorinated flakes are highly interactive with the surrounding environment and can be used for sensor applications.

5 Thermal Properties by Computational Simulations

Especially in nanotechnology, the thermal characteristics of new materials are critical to their design and performance. Composites made from integrating quantum dots into a matrix material are an exciting new field of study with numerous potential uses. By controlling their thermal behaviour, these composites can be optimised for a wide variety of uses. To investigate and comprehend these materials' thermal properties at the nanoscale, computational simulations are an indispensable tool. The importance of computational simulations in investigating the thermal characteristics of quantum dot-based composites is explored in this article. The ability of a substance to conduct heat is described by its thermal conductivity, a fundamental thermal attribute. The thermal conductivity of composites based on quantum dots is crucial because it has a direct impact on the performance of these materials in a wide range of contexts. Small size and quantum confinement factors mean that quantum dots can have dramatically different thermal conductivity from bulk materials. Computational simulations, and in particular Molecular Dynamics (MD) simulations, are used by scientists to learn about and make predictions about the thermal conductivity of these composites. In MD simulations, atom mobility and energy transmission within the composite are modelled and monitored. The thermal conductivity can be determined by measuring the atomic vibrations and energy transfer. These simulations can shed light on the impact of variables like quantum dot size and spacing, as well as dot-matrix interactions, on thermal transport inside the composite.

The quantity of heat energy needed to increase the temperature of a material by a given amount is known as its "specific heat capacity." Knowing the specific

heat capacity of composites based on quantum dots is crucial for uses in temperature regulation and management. The electrical structure of quantum dots and their interactions with the surrounding matrix can be predicted with the help of computational simulations, in particular Density Functional Theory (DFT). To determine the composite's specific heat capacity, this data is necessary. In addition, the lattice contribution to specific heat can be understood by classical MD simulations. Since the specific heat capacity of quantum dots is highly application-dependent, computational simulations are an invaluable tool for optimising this feature for particular uses. The term "thermal expansion" is used to refer to the enlargement or contraction of a substance as a result of changes in temperature. In order to design materials for specific applications, knowledge of thermal expansion is essential for quantum dot-based composites. The phonon frequencies and vibrational modes of both the quantum dots and the surrounding matrix can be predicted using computational simulations, in particular those using lattice dynamics and Density Functional Theory. The thermal expansion coefficient can be calculated by observing the motion of atoms within the composite when it is heated and cooled. Computational simulations allow us to probe and manipulate the thermal expansion of quantum dots by changing their size, shape, and interaction with the matrix material.

In addition to these standard thermal characteristics, composites based on quantum dots may display unusual behaviours as a result of quantum mechanical processes. The electrical and phonon structure of quantum dots, as well as their interactions with the surrounding matrix, can be understood with the help of computational tools like Density Functional Theory. Understanding the effects of quantum confinement and quantum dot size on heat transit and storage inside the composite requires an accurate assessment of its thermal properties, which may be achieved with the help of the data presented here. It is also important to carefully analyse the simulation boundary conditions you choose, such as periodic boundary requirements. To get reliable findings, one must carefully evaluate the simulation cell size and the impact of finite size effects on thermal parameters. In order to characterise temperature-dependent behaviour in quantum dot-based composites, it is necessary to run simulations over a wide temperature range. In addition, the thermal characteristics of the composite might be drastically altered by the presence of flaws or impurities. Researchers can evaluate the effects of these flaws on heat transport, thermal expansion, and specific heat capacity by extending computational models to include them. Knowing that these flaws can be present in practical applications is crucial for understanding how real-world materials behave. The examination of simulation data has also benefited from the use of machine learning methods. Conventional analytic approaches may miss trends, patterns, and correlations that can be uncovered by applying machine learning algorithms to simulation findings. These methods can be used to foretell how different quantum dot composites would behave at high temperatures. This will help in the design of novel materials with controllable thermal properties. Computational results must be compared with experimental observations to verify the reliability of simulations and improve the quality of the underlying models. Researchers can verify their models and increase their faith in their accuracy by comparing them

to experimental data, which provides the real-world context for the computational predictions.

Numerous promising applications could be realised with a better understanding of the ability to manipulate the thermal characteristics of composites based on quantum dots. For optimal performance in thermoelectric applications, for instance, it is essential to optimise heat conductivity while keeping good electrical conductivity. To keep devices from overheating and failing, effective heat management is crucial in nanoelectronics. By manipulating their thermal behaviour, composites can improve the efficacy of photothermal therapy in killing cancer cells. To better understand the physics at play and to direct the development of such materials with specific thermal characteristics, computational simulations play a crucial role.

In conclusion, the investigation of the thermal characteristics of composites based on quantum dots requires the use of computational models. Quantum mechanical effects, thermal conductivity, and specific heat capacity can all be better understood with the help of these models. Quantum dot size, flaws, and temperature dependence are all important considerations that contribute to a fuller picture of the nanoscale behaviour of these materials. Electronics and medicine are only two of the many fields that stand to benefit from advances in our capacity to anticipate and manipulate the thermal properties of composites based on quantum dots. Computational simulations are a crucial tool in the development of innovative materials, especially when combined with machine learning approaches and experimental validation.

The invention of thermoelectric materials with a high figure of merit is one of the most difficult tasks in modern material research. It has been suggested that a QDSL, which is made by clustering quantum dots into patterned composites, could be a good choice for thermoelectric applications due to its lower lattice thermal conductivities. Reduced lattice thermal conductivity due to acoustic phonon scatterings at the interface and the quantum confinement of carriers contribute to the enhancement of ZT in QDSL. By manipulating the cluster configurations, one can change the Fermi level positions and hence the electronic characteristics of QDSL. Thermoelectric figure-of-merit enhancement in quantum dot superlattices was proposed by Balandin et al. For the p-type regulated superlattice of Ge dots on Si, a proof-of-concept calculation has been performed. It is demonstrated that the thermoelectric figure-of-merit can be improved by tuning carrier transport in such structures, under circumstances for miniband formations, resulting in high carrier mobility, a high Seebeck coefficient, and a high thermoelectric performance. Maximising the gain requires setting the settings of the quantum dot superlattice so that the majority of the current flows in the wide, well-separated minibands [31].

6 Summary and Conclusions

This chapter provides a thorough examination of the theoretical underpinnings, sophisticated modelling approaches, and simulations required to comprehend quantum dot behaviour in multiphase systems. The unique electrical and optical

features that quantum dots, as nanoscale semiconductor structures, offer have tremendous potential in a wide range of applications. The confinement effects and energy level structures that characterise quantum dots' behaviour are introduced as the chapter's first topic of discussion. Next, it explores state-of-the-art modelling techniques, such as Density Functional Theory, that pave the road for reliable prediction of quantum dot properties. The effects of quantum dots on optical, electrical, and thermal characteristics are discussed, and their application to multiphase systems is investigated. This chapter uses state-of-the-art simulations to show how to develop and optimise systems based on quantum dots, with a focus on customising their properties for use in photonics, electronics, and renewable energy.

The importance of theory, modelling, and simulation in furthering our knowledge of quantum dots and their behaviour in multiphase systems is highlighted in this chapter. Quantum dots are interesting prospects for a wide range of applications due to their unique features, but a strong theoretical foundation is required to properly exploit their potential. Researchers can precisely foretell quantum dot features using Density Functional Theory and other state-of-the-art modelling approaches, paving the way for the custom construction of materials with desired electrical and optical properties. The interactions of quantum dots with other materials have a major effect on the characteristics of multiphase systems in which they are incorporated. This chapter highlights the significance of these interactions and the use of simulations in achieving optimal performance in quantum dot-based devices for use in next-generation photonics, electronics, and renewable energy applications. Finally, the book chapter offers helpful advice for scientists and engineers who want to use quantum dots in various applications, thereby boosting the fields of nanoscience and materials engineering.

QD modelling and simulation is set to become a rapidly developing and game-changing industry in the near future. Accurate and comprehensive simulations are in high demand as the number of potential uses for quantum dots in nanoelectronics, photonics, and quantum technologies increases. Researchers will be able to optimise QD features for specific uses if they can model them more accurately using cutting-edge quantum mechanical techniques. QDs' behaviour in different contexts can be better understood with the use of multiscale models that combine quantum mechanics and classical techniques. In addition, understanding the role that ligands and solvents play in the interactions between QDs and their environments is essential for developing QDs with desirable characteristics. Advanced materials and devices will be easier to create with the help of simulations as the use of QD assemblies, heterostructures, and complicated systems grows in popularity. Unlocking the full potential of quantum dots in developing technologies will require a multidimensional strategy, including the integration of machine learning techniques and an emphasis on examining environmental and toxicological concerns.

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Future Outlook and Present Market

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Abstract Composites containing quantum dots (QDs) have recently grown in importance, driving innovation in a wide range of sectors. This abstract explores the present and future market dynamics of composites based on quantum dots and the uses they have. With the increasing need for high-performance materials, QDs offer an unmatched opportunity to improve the electrical, optical, and catalytic characteristics of composites. Industries are investigating new uses in fields including electronics, healthcare, and energy, which have led to a boom in R&D efforts and the current state of the industry. When creating composites with specific functions in mind, quantum dots are an absolute must due to their size-tunable characteristics and quantum confinement effects. Composites based on quantum dots have the potential to shape the next generation of high-tech materials and technologies, which emphasizes the revolutionary effect of QDs on the field of materials research.

1 Background

Nanostructured materials are intriguing due to their ability to connect the macroscopic and molecular scales, opening up novel possibilities for applications, particularly in the fields of electronics, optoelectronics, and biology [1]. Nanostructures can be classified into three categories: (1) two-dimensional, such as thin films or quantum wells, (2) one-dimensional, such as quantum wires, and (3) zero-dimensional, also known as dots. In recent years, there has been significant interest in studying the optoelectronic characteristics of nanostructured semiconductors, namely quantum dots (Qdots). This is because many fundamental features of these materials are influenced by their size, particularly when it falls inside the nanometer range. A Qdot is considered to be zero-dimensional compared to the bulk material. Due to the limited amount of electrons, the density of states (DOS) for nonaggregated zero-dimensional objects exhibits discontinuous quantized energies [2]. (While the box is considered to be zero-dimensional in terms of its bulk, it is treated as a box in the field of quantum

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mechanics. The size of the box is significant and will be examined further.) Occasionally, the existence of a single electronic charge in the Qdots causes the avoidance of an additional charge and results in a stair-shaped I-V curve and DOS. The size of each step in the staircase is directly proportional to the inverse of the radius of the Qdots. The transition between the bulk, Qdot, and atomic characteristics of a material is determined by the composition and crystal structure of the compound or elemental solid. A wide array of fundamental qualities can be achieved by altering the size while maintaining a constant composition, and some of these properties are discussed. Quantum dots can be classified into two main categories: elemental systems and compound systems. This review focuses on compound semiconductor-based nanostructured materials and their diverse applications, which leverage their optoelectronic and optical capabilities.

QDs exhibit exceptional potential in diverse applications owing to their distinct optical and electrical characteristics. Nanocomposites including QDs, which entail the integration of QDs into composite materials, have garnered considerable interest due to their potential applications in various disciplines. In this chapter we will provide a speculative outlook for the potential development of nanocomposites, incorporating QDs, focusing on their design, fabrication procedure, and growing applications [3]. The distinctive optical features of QDs have rendered them unparalleled possibilities for imaging applications both in living organisms (in vivo) and in laboratory settings (in vitro). The optical qualities of the QDs arise from their inorganic core, which is accountable for the fundamental semiconducting capabilities and optical properties. Before capping a QD with ligands, it is common to passivate the active core surface with an additional inorganic shell [4]. The optical properties of QDs are enhanced by the larger band gap of the shell in comparison to the core material, effectively preventing the migration of electrons and holes into the shell [5]. QDs exhibit distinctive optical characteristics as a result of the confinement of electron–hole pairs (excitons) within the limits of the nanocrystal grains. The unique photo-physical characteristics of QDs, such as wide absorption spectra and precise emission spectra that can be adjusted by size and composition, along with their ability to emit light, high fluorescent efficiency, strong absorption capacity, resistance to fading under light exposure, large difference between absorption and emission wavelengths, and reduced intermittent fluorescence, have established them as suitable options for a wide range of biomedical uses [6].

2 Tailored Characteristics

Scientists are projected to prioritize the development of QDs with customized characteristics, including sizes, shape, and composition, to attain precise functionality [7–14]. Future designs of nanocomposites may use various quantum dot varieties or other nanomaterials to generate multifunctional nanocomposites that exhibit improved performance.

3 Fabrication Procedures

The focus should be on finding efficient and economical fabrication methods for QDs and their nanocomposites to enable mass production[15]. Nanofabrication techniques, such as molecular self-assembly and template-assisted procedures, can allow accurate control of the arrangement of QDs within nanocomposite structures [16–18].

4 Emerging Applications

QDs possess exceptional optical characteristics, making them suitable for food industry and medicinal purposes such as improved imaging and tailored therapy[19–22]. Future advancements could encompass the development of more effective methods for administering drugs and the improvement of contrast agents.

Quantum dot-based displays have already been introduced in the market, providing enhanced colour accuracy and brightness compared to conventional displays [23]. Continuing research has the potential to result in display technologies that are even more efficient in terms of energy consumption and produce more vivid visuals.

QDs have the potential to be used in very effective energy harvesting devices, such as solar cells. Research may concentrate on maximising the efficacy of energy conversion and investigating innovative methods for harvesting solar energy[24].

5 Environmental Sensing

Sensor Technologies: Nanocomposites based on QDs have the potential to be utilised in the development of advanced sensor technologies for the purpose of monitoring the environment, identifying contaminants, and assuring the safety of atmospheric and water resources [25–27].

Smart Materials: The incorporation of QDs into smart materials may make it possible to create materials that are responsive and able to adjust to the shifting conditions of their surrounding environment[28].

6 Optical Computing

QDs provide distinctive optical properties that can be utilised to enhance optical computing, leading to improved speed and efficiency in computational solutions.

7 Regulatory and Safety Considerations

To address any environmental and health risks that are linked with the utilization of these nanocomposites, there may be an increased emphasis placed on the establishment of safety standards and regulations as quantum dot applications grow more widespread.

The future of QDs-based nanocomposites depends on ongoing scientific advancements, technical advances, and the effective transition of laboratory-scale progress into real-world applications[29, 30]. The potential of QDs in diverse fields can only be realized by continuous multidisciplinary collaboration among materials scientists, physicists, biologists, and engineers.

QDs have become widely adopted in the display industry [31]. Quantum dot displays, commonly referred to as QLED displays, are being integrated into televisions, monitors, and other consumer electronic products. These displays have benefits such as higher colour accuracy, expanded colour range, and increased brightness in comparison to conventional LED displays [32]. QDs are now being investigated for their potential use in a variety of biomedical applications, including diagnostics and imaging. As a result of their one-of-a-kind optical features, QDs are extremely useful for fluorescence imaging in biological systems. Investigations are also being conducted into applications in the fields of drug delivery and theranostics, which refers to combination diagnosis and therapy.

Ongoing research and development efforts are centred on enhancing the performance and stability of QDs, investigating new techniques of synthesis, and broadening the scope of applications for these QDs [33]. There is a lot of competition in the market for QDs, and many different companies and research organizations are actively contributing to the growth of quantum dot technology. The companies considered to be significant participants in this industry include well-established technological companies and newer startups that focus on nanotechnology and materials science [34]. Anticipated advancements and partnerships are expected to propel the use of QDs in many industries as the market for this technology progresses. The future trajectory of the QDs industry will be significantly influenced by the development of production methods that are both scalable and cost-effective, as well as the resolution of environmental and safety concerns.

QDs based nanocomposites have been actively investigated in a wide variety of practical research domains ever since their discovery. These fields include energy to electromagnetic interference (EMI) and catalysis to sensing, tribology, and biomedicine. However, despite their widespread application, they continue to have bottlenecks and drawbacks that need to be resolved and overcome in order to realize their full potential in the applications that were described earlier [35]. Every one of these applications has the potential to benefit from the advantages of the synergistic effect that is brought about by the rational design of functional zero dimensional materials, as well as surface-modified and hybrids based on mixed-dimensional materials properties [36].

8 QDs in the Healthcare Market

The global market for quantum dots (QDs) is now valued at USD 4 billion in 2021 and is projected to reach USD 8.6 billion during the next five years [37]. While display devices and LED applications have been the main focus of the business, there has been a growing interest in the biomedical uses of quantum dots (QDs).The user's text is "Citation83". Within this particular section, our aim is to emphasise the prominent market participants and their notable advancements in utilising quantum dots for biological purposes [38]. NANOCOTM holds a significant position in the healthcare market for quantum dots (QDs), with a projected market size of USD 1 billion in 2022 as per the company's prediction. HEATWAVETM is one of NANOCO's commercially available products that utilises quantum dots (QDs) technology for medical biosensor applications. This instrument can be utilised across a wide electromagnetic spectrum (400–1650 nm) to accurately measure several proteins in human blood, such as haemoglobin (at 575 nm), bilirubin (at 455 nm), and glucose (at 1650 nm), without the need for intrusive procedures. VIVODOTS® is a commercial product that utilises quantum dots (QDs) for the purpose of mapping tumorous tissues during surgery and preventing the removal of healthy tissues unnecessarily. Abdellatif led a Phase I clinical experiment where carboxylic acid-functionalized CdS/ZnS core–shell type quantum dots (QDs-COOH) were linked to veldoreotide, a specific ligand for somatostatin receptors [39]. The quantum dots (QDs) were included into a topical cream to explore their potential for bioimaging and as a treatment for breast and skin malignancies. Stability concerns encompass the significant susceptibility to deterioration and clumping that may occur during storage. Industrial problems occur due to the intricate scaling processes and the environmental risks posed by the heavy metal constituents. Issues encountered in vivo include the significant reduction of the administered dose through renal clearance due to the extremely small particle size, lack of specificity due to non-specific interactions with tissues and cellular membranes, and toxicity within cells caused by the production of reactive oxygen species (ROS) or damage to DNA.

From a pharmacological perspective, quantum dots (QDs) are extremely small colloidal particles with a large surface area and metallic properties. This makes them prone to aggregation, deterioration, hygroscopicity, and chemical redox changes. Even little alterations in the physico-chemical characteristics of quantum dots (QDs) can have a significant effect on their optical qualities [40]. Considerable endeavours have been made to enhance the physical and chemical stability of quantum dots (QDs). Zhang et al. introduced a chemical technique to stabilise PbS quantum dots (QDs) that are prone to degradation due to the oxidation of chalcogen atoms on their surface. A surface reaction using trioctylphosphine was employed to passivate surface S atoms with lead mono-carboxylate. The proposed technique successfully shielded quantum dots (QDs) from oxygen and enhanced their quantum characteristics, regardless of the particle size or the initial surface ligands [41]. CdSe/ZnCdS core/shell quantum dots (QDs) and InP/ZnSeS core/shell QDs were shielded against high temperatures and oxidation by applying a double polymeric shell made

of thiol-terminated poly(methyl methacrylate-b-glycidyl methacrylate) (P(MMA-b-GMA)-SH) block copolymer ligands. The external layer consisted of clear PMMA, which did not affect the optical characteristics of QDs. Meanwhile, the inside shell, which was cross-linked, provided protection against oxidation [42]. Another issue that emerges due to the low solubility in water is the use of organometallic synthesis, which is a very efficient and often employed process for producing quantum dots (QDs). Proposed solutions include replacing hydrophobic ligands on the surface with hydrophilic thiol-containing compounds, applying hydrophilic shells as a coating, and utilising aqueous-based production [3].

9 Summary

In the past decade, numerous synthesis techniques and tactics have been developed to improve the properties of quantum dots (QDs) for specific applications. This class of materials has lately demonstrated significant promise in various biomedical applications, including cancer therapeutics, tissue engineering, and regenerative medicine. Moreover, quantum dots (QDs) have lately been discovered to be valuable instruments in particular scientific domains, including the simultaneous administration of drugs to two targets in real-time and the visualisation of adipose regions in obese individuals by molecular imaging. QDs have a very wide surface area and a unique chemical structure, which allows them to successfully interact with various biomolecules. These interactions can be crucial occurrences that impact the ultimate destiny of both specialised and undifferentiated cells in the human body. The utilisation of quantum dots (QDs) in cancer care has garnered significant attention due to their notable contribution to the advancement of chemotherapy and protein-based medicines. Furthermore, quantum dots (QDs) possess optical properties specifically designed for bioimaging and biosensing, making them potentially transformative in the field of medicine. In recent years, the primary obstacle has been the severe and prolonged toxicity of quantum dots (QDs) in biological organisms. The primary organs impacted by the accumulation and resulting toxicity of QDs are the lungs, kidneys, and liver. However, the brain has been found to be more resilient to the toxic effects of QDs. This discovery encourages further experimentation to assess the potential of different types of QDs in the treatment and imaging of neuroregenerative diseases.

Through the use of chemical, physical, and biological methodologies, together with revolutionary strategies such as surface functionalization approaches, it is anticipated that quantum dots (QDs) may soon find practical applications in therapeutic settings. The primary objective of these approaches should be to address the persistent toxicity of quantum dots (QDs) in order to develop a pharmaceutical QD product suitable for clinical and industrial use. Additional difficulties may arise from the need to achieve consistent results in the production of quantum dots (QDs) and the establishment of rigorous quality control measures to minimise discrepancies between different batches. Furthermore, it is crucial to thoroughly evaluate and assess the

administration routes and protocols for introducing the manufactured quantum dots (QDs) into the body to ensure compliance with the necessary clinical standards. Recent advancements in our understanding of molecular interactions within the body have revealed the promise of using quantum dots (QDs) for personalised medicine. However, the issue of QDs binding to molecular compartments (such as proteins) in cells and tissues without specificity still poses a challenge. Researchers worldwide are urged to focus greater attention on this matter. An further crucial concern about the widespread use of quantum dots (QDs) pertains to the inconsistencies in the structure and composition of QDs, whether they are in their bare form or functionalized. These variabilities pose challenges in reaching definitive conclusions about the potential of QDs in theranostic applications. Further study is necessary to establish dependable techniques. While the issues surrounding the optimal use of QDs in clinical applications have not been completely resolved, recent advancements have significantly propelled these materials towards their therapeutic potential.

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This book delves into the delicate realm of neurodegenerative illnesses, navigating the vast landscape of molecular targets with care and purpose. Researchers are studying the complex pathways involved in diseases such as Alzheimer's, Parkinson's, and Huntington's in order to identify specific molecules that could be targeted for therapy. The present work explores potential methods of intervention by carefully analysing neural circuits, protein misfolding, and genetic predispositions, unravelling the complexities of the human mind by focusing on individual molecular targets. As new findings emerge, reducing the severe consequences of neurodegenerative illnesses becomes increasingly possible, providing optimism for millions of people throughout the world.

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Prashant Tiwari
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CHAPTER 6

MICRORNA-BASED MAPPING OF NEURODEGENERATIVE DISORDERS

HARIPRASAD M.G[1],
MOQBEL ALI MOQBEL REDHWAN[1],
AND SOURAV GUHA[1]

Abstract

MicroRNAs are pivotal modulators of gene expression, governing numerous cellular pathways through their role in post-transcriptional regulation. These small, non-coding RNA sequences fine-tune the translation of genetic information into protein synthesis, a process crucial for maintaining neuronal health and function. The dysregulation of miRNAs is increasingly implicated in neurodegenerative disorders, providing insights into the molecular underpinnings of diseases such as Alzheimer's, Parkinson's, and Huntington's disease. This text provides a detailed examination of miRNA biogenesis, their mechanism of action in gene silencing, and the pathological consequences of their aberrant expression in the nervous system. This study also explores the emerging significance of miRNAs as biomarkers for early detection and prognosis, alongside their potential as novel therapeutic targets. Advances in the computational prediction of miRNA targets are discussed, highlighting the synergy between bioinformatics and experimental research. Furthermore, the text reviews current advances and obstacles in miRNA-based therapeutic approaches, including RNA interference, focusing on their translational potential in clinical settings. By illuminating the role of miRNAs in

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neurodegeneration, this analysis emphasizes their substantial promise in revolutionizing the diagnosis and treatment of neurodegenerative disorders.

Keywords: MicroRNAs (miRNAs), Neurodegenerative diseases, Alzheimer's disease, Gene expression, Biomarkers, RNA interference.

Introduction

MicroRNAs, or miRNAs, are small RNA molecules typically comprising 21 - 25 nucleotides. These molecules are known not to code for proteins, but rather, to regulate a variety of crucial biological processes. These processes include developmental timing, cell division, asymmetrical cell development, formation of dendritic spines (which are essential for nerve cell connections), early stages of embryo development, growth of tumours , differentiation of stem cells, and the body's defense against viruses. Their role is not only fundamental in normal physiological processes but also in disease mechanisms, especially those involving oxidative stress [1]. Oxidative stress describes a state in which there is a harmful surplus of free radicals—reactive molecules that cause cell damage—and a lack of sufficient antioxidants to counteract them. This imbalance can lead to cellular harm, especially in nerve cells, which can result in various neurodegenerative conditions. A landmark discovery in 1998 by Fire *et al* demonstrated that double-stranded RNA molecules could silence genes through a process known as RNA interference. Subsequently , in 1999, Baulcombe's research showed that the miRNA family, including let-7miRNA, is involved in gene silencing in animals and plants. This highlighted the ubiquitous presence of miRNAs in different life forms [2-4].

For instance, miR-31 is known to interact with the gene BCL6 and is thought to help reduce cell damage by inhibiting the expression of polycystin 1 (PKD1). Evidence suggests that blocking BCL6 can help protect nerve cells from oxidative stress by targeting the miR-31/PKD1 pathway [5]. Another miRNA, miR142a-5p, affects mitochondria, leading to dysfunction and cellular self-digestion processes that result in muscle atrophy when nerves are damaged [6].

The importance of miRNAs is also recognised in the context of neurodegenerative diseases, which are conditions that typically worsen over time and pose a significant health threat. The World Health Organization forecasts that by 2040, certain illnesses may overtake cancer as the world's

second primary cause of mortality. This is a major concern because the human nervous system has limited regenerative capacity, making such diseases largely irreversible [7,8].

Degenerative neurological conditions, progressively impair cognitive and motor functions, often due to neuronal loss. Such conditions are increasingly attributed to changes in protein-coding genes caused by somatic mutations that accumulate over time. Remarkably, noncoding RNAs, which constitute the majority of cellular RNAs, are key players in the regulation of protein synthesis, underscoring the importance of understanding mRNA dynamics [9-10].

Inflammation is a common thread in the pathophysiology of neurodegenerative diseases. Long-term, uncontrolled stimulation of the innate immune system can lead to brain damage. Macrophages, a type of immune cell, can adopt different states called M1 and M2 phenotypes, with each playing distinct roles in inflammation and disease progression [11-13]. Certain miRNAs influenced these macrophage states, potentially offering new avenues for the treatment of inflammation-related diseases. Metabolic dysfunction is another contributor to neurodegenerative conditions such as Alzheimer's (AD) disease, marked by the build-up of amyloid-beta plaques [14,15]. The altered metabolism of neurotransmitters is also implicated in disease progression. For example, a reduction in miR-96-5p in AD models has been linked to changes in amyloid-beta ratios through the regulation of specific genes involved in amyloid-beta production [16]. Finally, miRNAs are implicated in excitotoxicity, a harmful process triggered by excessive levels of the neurotransmitter glutamate, leading to neuronal damage and death. Specific miRNAs that regulate glutamate receptors or transporters, are crucial for maintaining appropriate glutamate levels and preventing neuronal damage.

As research on miRNAs continues to deepen, their roles in diseases, especially neurodegenerative disorders, become clearer, offering potential for new biomarkers and therapeutic targets. miRBase, a comprehensive miRNA database, aids researchers in this endeavor, by providing an extensive catalogue of known miRNAs and their gene targets. However, despite these advances, the detailed functions, and regulatory mechanisms of miRNAs in the development of degenerative neurological conditions remain an active and critical area of study.

Mechanism of miRNA

MicroRNA genes are predominantly transcribed by RNA polymerase II within the cell nucleus into primary miRNAs. In some instances, RNA polymerase III also participate in this process [17]. These pri-miRNAs are characterized by stem-loop structures that culminate in a hairpin turn and can extend several hundred nucleotides in length, with partially complementary sequences along the stem portion.

The transformation of pri-miRNA into a shorter, precursor form (pre-miRNA) is facilitated by the microprocessor complex, which includes the essential RNase III enzyme Drosha and the DGCR8 protein [18,19]. Drosha precisely trims the pri-miRNA at the base of its 5′ and 3′ arms, whereas DGCR8 identifies the exact sites for cleavage [17]. As a result, a pre-miRNA molecule of approximately 70 nucleotides (nt) in length is produced [20].

Once processed, the pre-miRNA is bound via XPO5 and conveyed out of the nucleus into the cytoplasm via the Ran-GTP complex, passing through the nuclear pore complex [17,18]. In the cytoplasm, Dicer endoribonuclease excises the loop of the pre-miRNA, converting it into a mature miRNA duplex, typically 19–24 nt long. This mature miRNA subsequently plays a role in the regulation of gene expression at the post-transcriptional level [19,20].

Gene Silencing at the Post-Transcriptional Level Mediated by miRNAs

Post-transcriptional gene silencing (PTGS) is mediated by a specialized ribonucleoprotein known as the miRNA-induced silencing complex (miRISC). This complex is integral to the process and consists of a single-stranded miRNA and various protein components that make up the RISC loading complex, which includes the Dicer enzyme, TRBP, and Argonaute-2 protein [17,20].

Dicer, a member of the RNase III enzyme family, cleaves double-stranded RNA (dsRNA) into smaller RNA fragments. TRBP, with its dsRNA-binding domain, enhances the cleavage activity of Dicer by stabilizing it. Although TRBP is not strictly required for PTGS, its presence boosts the efficiency of the process. In addition , TRBP is involved in the recruitment of Ago2, which is a core component of the RISC loading complex [17,21]. Within

miRISC, the mature miRNA strand that guides the complex to the mRNA target is typically selected on the basis of its lower thermodynamic stability. This guide strand is the principal agent in directing the silencing complex to mRNA, whereas the opposite passenger strand is often degraded [18,19].

Upon locating the target mRNA's 3′UTR, miRISC can either inhibit translation or cleave the mRNA. The "seed region" of the miRNA, typically found at positions 2-8 from the 5' end, is crucial for this process because of its near-perfect base pairing with the mRNA's 3′ UTR [17]. Ago2 plays a pivotal role in translational repression by competing with the eIF4E initiation factor for binding to the mRNA cap structure. Other suggested mechanisms of translational repression by miRNAs include hindering the association of ribosomal subunits, inducing premature termination of polypeptide synthesis, and promoting degradation of the nascent polypeptide chain [21].

Furthermore, miRNA can facilitate mRNA decay through two mechanisms. The first involves direct cleavage of the mRNA by the endonucleolytic activity of Ago2 at the site of miRNA-mRNA base pairing. The second mechanism involves miRNA directing the removal of the 3' poly(A) tail, leading to mRNA degradation through exonucleolytic digestion from the 5' end [19,22].

Identifying miRNA Targets: Overview of Computational Tools

Computational methods have significantly advanced our ability to identify miRNA targets. These methods provide a quick and efficient approach for investigating miRNAs and their roles in various diseases, facilitated by their interactions with multiple mRNA targets. Among the leading tools in this domain are RNA22, TargetScan, miRbase, miRanda, and PicTar, each of which employssophisticated algorithms to detect potential miRNA:mRNA interactions within miRNA sequence databases [33,34].

TargetScan, the pioneering algorithm in this field, utilizes features such as Pct scores, context+ scores, and TargetScanS to refine prediction accuracy. It assesses the likelihood of gene targets for a given miRNA across different species, including humans, mice, and zebrafish, prioritizing results based on the highest probability of interaction as determined by Pct scores, conservation, and binding site analysis [34–36].

The miRanda algorithm facilitates the identification of genes involved in endogenous miRNA gene regulation. This tool employs a robust classification system for predicting miRNA:mRNA interactions [34-37].

MiRBase is a comprehensive online database that offers a wealth of information on miRNA nomenclature, sequence data, and annotated target sites. It is particularly useful for bioinformaticians because of its extensive collection of miRNA sequences organized by related genomes [39].

PicTar specializes in predicting miRNA target sites within the human and mouse genomes [40], while PITA (probability of interaction by target accessibility) introduces a unique approach. It scans the untranslated region (UTR) for potential miRNA targets using a seed-matching tool and scores the sites accordingly [40,41].

Although each tool has a unique methodology for making predictions, using them in tandem can help narrow down potential targets, leading to more reliable experimental validations. Understanding the biological mechanisms underlying each tool is crucial, as this knowledge will guide researchers in selecting the most suitable tool for their specific research needs, balancing accuracy, and minimizing false positives and negatives.

miRNAs in Alzheimer's disease

The widespread occurrence of AD among the elderly is approximately 60%, and is characterisedby neuronal tissue loss, memory impairment, cognitive dysfunction, and hindered learning abilities. Despite extensive research, the definitive cause of AD remains elusive. However, several biomarkers have been strongly linked to the disease [42].

Tau protein is one such biomarker that is, involved in vesicle transport within cells. AD is marked by the hyperphosphorylation of tau, leading to its detachment from its usual binding partners and subsequent aggregation with other tau molecules, forming neurofibrillary tangles, that,impair neuronal communication. Amyloid-β is a derivative resulting from the cleavage of amyloid-beta precursor protein (APP). It tends to accumulate and form plaques in the brains of AD patients, contributing to the disease pathology [43]. Recent advances have identified microRNAs (miRNAs) as another set of biomarkers in AD. These tiny miRNAs, which do not code for proteins, and are present in both blood and cerebrospinal fluid, have been implicated in AD's pathogenesis. MiRNAs such as mir-9, mir-29, and mir-181 have been consistently observed in AD cases, suggesting their

involvement in inflammatory and immune reactions [44]. miRNAs can regulate amyloid-β levels, where mir-15 influences the rate of neuronal death by altering BCL2 levels, and mir-34 suppresses BCL2 translation, influencing apoptosis [43].

Furthermore, mir-128 has been implicated in tau pathology by affecting the synthesis of BAG2, which also influences neuronal apoptosis. In addition, alterations in miRNAs are associated with changes in tau accumulation [43]. The miR-15 family has been shown to regulate tau phosphorylation, which is a process that modifies tau protein function and aggregation. MiRNAs also modulate tau metabolism, with specific miRNAs such asmir-219 playing a role in both tau toxicity and regulation at the posttranscriptional level [45].

In addition to their role in protein regulation, certain miRNAs are involved in memory deficits linked to neurodegenerative diseases. For instance, overexpression of mir-132, may lead to impaired memory in AD due to increased neuronal death associated with elevated FOXO3a levels. In the context of synaptic plasticity, mir-124 interactswith Egr1 and AMPAR GluA2, both of which are crucial for maintaining cognitive function. The mir-34 family affects neuronal signallingby regulating proteins such as AMPAR and mGLUR7, with mir-34c being linked to memory impairment and neurodegeneration [46].

Therapeutically, miRNAs present a novel target for AD intervention. Specific miRNAs that regulate BACE-1, an enzyme involved in amyloid-ß production, have been identified.. Adjusting the levels of these miRNAs may modulate BACE-1 activity, potentially reducing amyloidosis. The use of synthetic miRNAs, including antisense strands and those attached to facilitators such as cholesterol for better cellular uptake, could restore the levels of miRNAs that are downregulated in AD [47].

The relationships between miRNAs and their molecular targets, and their role in AD pathogenesis, are outlined in Figure 1 and summarized in Table 1.

Table 1 lists various miRNAs with differentconcentrations in AD patients. Some miRNAs have confirmed targets involved in AD's neuronal pathology. Others show changes in blood, spinal fluid, or brain tissue in AD but lack validated target research to clarify their role in disease mechanisms.

miRNA	Level Changes	Molecular Targets	References
miR-let-7f-5p	Downregulated	Caspase 3	[48]
miR-124	Downregulated	BACE1	[49]
miR-15b	Downregulated	BACE1 and APP	[50]
miR-219	Downregulated	Tau or MAPT (Microtubule-associated protein Tau)	[51]
miR-214	Downregulated	Atg12	[52]
miR-342-3p	Upregulated	Activation of JNK-MAPK cascade	[53,54]
miR-206	Upregulated	BDNF	[55]
miR106-B	Upregulated	Fyn	[56,57]
miR-9	Upregulated	APP, UBE4B	[58,59]
miR-330	Upregulated	VAV1 via the MPAK pathway	[60]
miR-455-3p	Upregulated	APP, NGF USP25, PDRG1 SMAD4,UBQLN1,SMAD2,TP73, VMAP2, HSPBAP1and NRXN1	[61]
miR-195	Upregulated	BACE 1	[62]
miR-188	Downregulated	BACE1	[63,64]
miR-200	Upregulated	S6 Kinase B1, mTOR	[65]
miR-128	Upregulated	PPAR-γ	[66]
miR-127-3p	Downregulated		[53]
miR-146a	Upregulated	NF-kB	[67,68]
miR-30a-5p	Upregulated	BDNF	[69]
miR-93	Downregulated		[70]
miR-34a	Upregulated	ADAM10, NMDAR2B and SIRT1	[71,72]

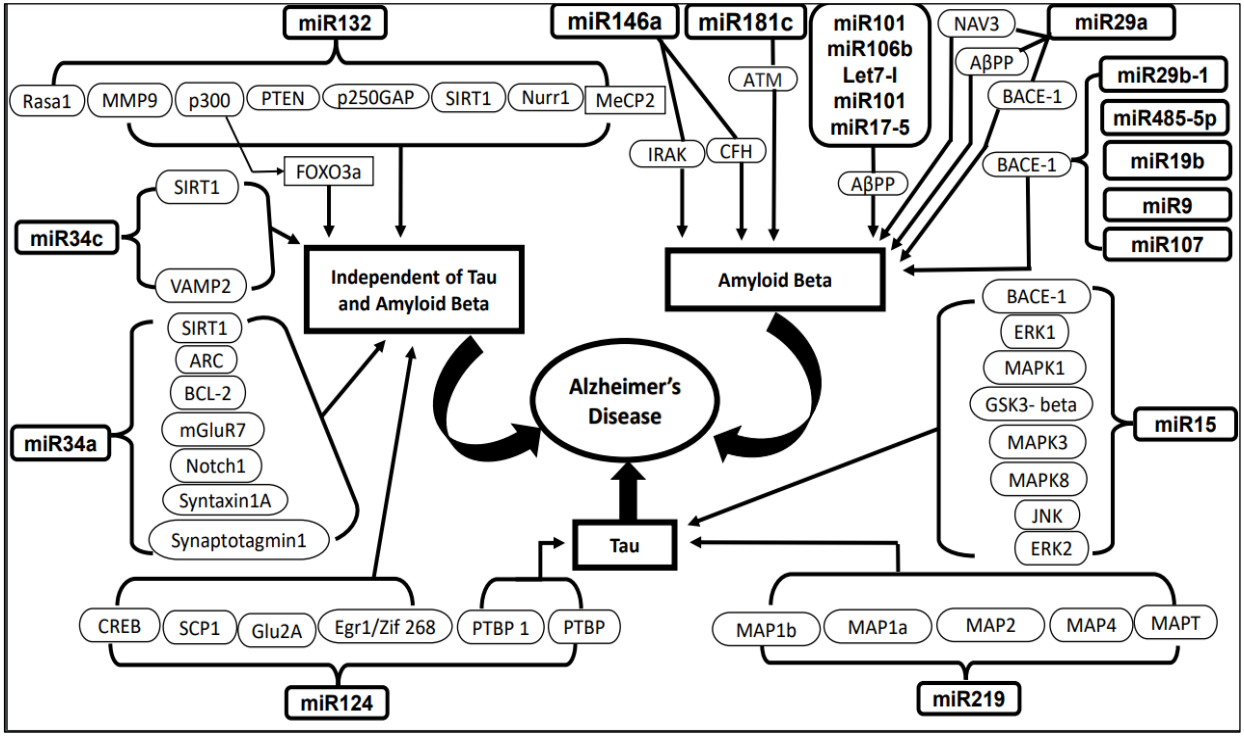

Figure 1 illustrates how certain miRNAs influence AD by interacting with tau and amyloid-beta (Aβ) proteins, and by affecting other cell processes that contribute to nerve cell damage.

Parkinson's Disease

Parkinson's disease (PD) is recognized as the second most prevalent neurodegenerative disorder, impacting approximately1% of individuals aged 60 and above. Characterized by progressive deterioration in motor skills, this disease is primarily caused by the loss of dopamine-producing neurons in the substantia nigra. As PD progresses, neurodegeneration extends to other areas of the brain, leading to symptoms such as dementia and psychosis.

PD presents with a range of clinical symptoms, notably including bradykinesia, characterized by a noticeable slowness in movement. Patients often experience muscular rigidity and a distinctive resting tremor. As the disease progresses, individuals may also encounter challenges in maintaining balance and postural stability. The causeof PD is complex and involves both genetic and environmental elements. A critical pathological feature of PD is the formation of Lewy bodies within neurons. These are aggregates primarily composedof alpha-synuclein, neurofilament, and ubiquitin proteins. While there is currently no definitive cure for Parkinson's disease, available treatments are mainly aimed at providing relief from the more debilitating symptoms.

One of the significant challenges in PD research is the absence of reliable diagnostic and prognostic methods, including distinct biomarkers for early detection. Studies have indicated that miRNAs may hold potential as biomarkers for PD. Changes in miRNA expression have been detected in body fluids [73–77] and brain tissue [78–83] of patients with PD as opposed to healthy individuals. Despite this, there is a lack of consistency in the results obtained from the different studies.

Investigations into miRNA expression have concentrated on the substantia nigra, which correlateswith the primary motor symptoms of PD. For example, Cho *et al*. [80] reported a significant reduction in miR-205 levels in the frontal cortex of individuals with PD. Furthermore, miR-133b, which is, crucial for the development and functioning of midbrain dopaminergic neurons, shows a significant deficiency in the substantia nigra of those affected by PD [78]. In addition, there has been a documented reduction in the levels of miR-34b and miR-34c in the same region [79]. The field also reports varying findings regarding the regulation of different miRNAs in PD brains, contributing to the intricate role of miRNAs in the pathology of PD [80, 82, 83].

The pathogenesis of PD may involve miRNA-mediated mechanisms becausemiRNAs have been identified to bind to key PD-associated genes such as SNCA and LRRK2. SNCA is prevalent in the presynaptic terminals of the brain [84], and its expression is inhibited by miR-7 and miR-153 [85]. LRRK2, critical for neuronal development [86], is directly inhibited by miR-205, as reported *in vitro* [80]. Moreover, FGF20, a neurotrophic factor crucial for the survival of dopaminergic neurons and highly expressed in the substantia nigra, has been linked to PD through SNPs in its 3´-UTR region [87,88].

Huntington's Disease

Huntington's disease (HD) is a prominent hereditary neurodegenerative condition characterized by abnormal elongation of polyglutamine chains. This genetic alteration manifests as a combination of motor, psychiatric, and cognitive disturbances [89]. A significant observation in HD is the reduction of specific microRNAs, namely miR-9 and miR-9*, in the cerebral cortex of affected individuals. This decrease becomes more pronounced as the disease progresses [90]. These microRNAs play a vital role by targeting elements of the REST complex. Specifically, miR-9 targets REST, whereasmiR-9* impacts CoREST. This localization is partly maintained through its interaction with huntingtin. However, in HD, the mutated huntingtin protein, characterized by its extended polyglutamine tract, disrupts this interaction [91]. Consequently, REST migrates to the nucleus, leading to the suppression of neuronal genes. This process is thought to contribute to the death of neuronal cells in HD.

The reduced expression of miR-9 and miR-9* in the HD-affected brain could thus result in an elevated REST protein level, facilitating its entry into the nucleus and worseningneurodegeneration. Intriguingly, the regulation between miR-9/miR-9* and REST is bidirectional. The promoter region of the miR-9/miR-9* gene contains an RE1 sequence and is subject to repression by REST. Therefore, the nuclear translocation of REST in HD explains the observed decrease in miR-9/miR-9*, which in turn may lead to an upsurge in REST/CoREST expression. This upregulation is harmfulto neurons, establishing a vicious cycle that accelerates the neurodegenerative process in HD.

In addition, miR-132 is another microRNA implicated in the pathology of HD, showing reduced expression in both HD mouse models and post-mortemhuman HD brains [92]. This decrease in miR-132 increases its target

mRNA, p250GAP [93]. The identification of further mRNA targets of miR-9/miR-9* and miR-132 has the potential to deepen our understanding of the molecular underpinnings of HD.

Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder that primarily affects neurons responsible for voluntary muscle movements. This disease causes gradual weakening and shrinking of muscles, leading to paralysis and, eventually, respiratory failure. Typically, ALS is fatal within three to five years after the first symptoms appear. Although ALS is a rare condition, ithas a profound impact on nerve cells in both the brain and spinal cord. The exact mechanisms underlying its development are complex and not fully understood.

Research into miRNAs has shown promise in understanding ALS pathology. This study involved examining the miRNA profiles of 12 individuals with ALS and 8 without the condition (controls). The results showed a significant increase in the levels of miR-338-3p in the ALS group. Conversely, a notable decrease was observed in the levels of several other miRNAs, including miR-451 and miR-1275, among patients with ALScompared with the control group [94]. A distinctive inflammatory miRNA signature in monocytes from ALS patients, correlated with findings in ALS mouse models [95]. Takahashi *et al*. [96] proposed miR-4649-5p and miR-4299 as potential biomarkers for ALS, following a study comparing miRNA levels in plasma from patients with ALSand controls.

A widespread decrease in miRNA in the spinal cords of patients with ALS [97]. Notably, 246 miRNAs were downregulated and, only 10 were upregulated. Some miRNAs, such as miR-146a*, were implicated in the regulation of neurofilament mRNA, suggesting a role in the pathogenesis of the disease. Koval *et al*. [98] reported dysregulated miRNA expression in both rodent models of ALS and human spinal cords, with miR-155 showing a significant increase. The inhibition of miR-155 by anti-miRNA oligonucleotides indicated a potential therapeutic avenue.

Williams *et al*. [99] highlighted miR-206 as an influential factor inALS progression. In mouse studies, miR-206 appearedto slow disease progression by enhancing the regeneration of neuromuscular synapses, suggesting its role as a modifier in ALS pathogenesis and a potential target for therapeutic strategies. This evidence underscores the potential of

miRNAs for early detection, diagnosis, prognosis, and even as therapeutic targets in ALS.

Spinal Muscular Atrophy

Spinal muscular atrophy (SMA), a genetic neuromuscular disorder, primarily arises due to a deletion in the SMN1 gene, affecting approximately 95% of SMA cases. This deletion leads to a shortage of the SMN protein, which is essential for the health of lower motor neurons . As a result, patients experience progressive weakening of muscles in the limbs and trunk. Currently, there is no definitive cure for SMA, but emerging therapies offer hope. One key focus in SMA research is the discovery of non-invasive biomarkers to track disease progression. Recent studies have highlighted the critical role of miRNAs in maintaining the health of post-mitotic spinal motor neurons . Impairments in miRNA function are linked to SMA symptoms, such as altered structure of neuromuscular junctions, and muscle fibre deterioration. Furthermore, there is a noticeable increase in the neurofilament (NFL) heavy subunit in miRNA-deficient spinal motor neurons , a marker commonly associated with motor neurone degeneration [100].

MiRNAs play a critical role in various aspects of neuronal functioning, including the development of neurones , formation of synapses, regulation of neurotransmitter release, and growth of axons. A study by Catapano *et al* [101] observed alterations in the levels of specific miRNAs (miR-9 , and miR-132)in the spinal cord and skeletal muscle with SMA and human patients with the same condition. Additionally, research has indicated an elevation in the levels of miR-183 in cortical neurons deficient in the SMN protein. This finding suggests a potential role of the SMN protein in modulating axonal translation via the miR-183/mammalian target of the rapamycin (mTOR) pathway [102]. MiR-206 plays a crucial role in the reinnervation process and could slow down the progression of SMA. This microRNA is also involved in the development of amyotrophic lateral sclerosis (ALS). Studies in ALS mouse models have shown that muscles less susceptible to denervation have increased miR-206 levels . This finding indicates the potential of miR-206 in promoting reinnervation and restoring neuromuscular junctions following nerve damage, highlighting its promise as a therapeutic target in treating neuromuscular diseases. Further research is essential to understand its full potential [103,104].

Friedreich's ataxia

Friedreich's ataxia (FRDA), a neurodegenerative condition, follows an autosomal recessive inheritance pattern. This disorder is characterised by a gradual decline in coordination abilities and isreferred to as ataxia. In addition , it is associated with hypertrophic cardiomyopathy, a condition in which the heart muscle thickens and functions improperly. As the most prevalent hereditary ataxia, FRDA has an occurrence rate of approximately 1–2 per 50,000 people, with carrier frequencies varying between 1:50 and 1:100.

The genetic basis of FRDA is linked to a specific GAA trinucleotide repeat expansion mutation within the first intron of the frataxin gene (FXN) on chromosome 9q21.1 [105]. Normal individuals typically have fewer than 43 GAA repeats, whereas those with FRDA have expansions ranging from several hundred to 2000 repeats, with 600 to 900 repeats being the most common [106]. This mutation leads to a substantial decrease in the production of frataxin, a crucial protein found throughout the body and involved in mitochondrial function [107]. The most affected tissues include sensory neurons in the spinal cord's dorsal root ganglia, heart, and pancreas [108]. In the spinal cord, degeneration of the posterior column manifests as progressive ataxia characteristic of the disease [109].

Research in the field is currently focusing on the possible impact of epigenetic modifications on FRDA, with a special emphasis on the role of miRNAs in these changes. Studies have discovered alterations in the 3′-untranslated region (3'-UTR) of the FXN gene, which are significant in FRDA. These modifications create new binding sites for miRNAs, which influence the expression of frataxin, a crucial protein in FRDA [110]. Moreover, changes in the AGTR1 gene, known to affect the cardiac aspects of FRDA, modify the binding site for miR-155, subsequently affecting AGTR1 expression [111,112]. Comparative analysis between patients with FRDA and healthy individuals has shown an imbalance in various miRNAs. Of particular interest, miR-886-3p is markedly elevated in patients with FRDA . Research indicates that targeting miR-886-3p can increase both the mRNA and protein levels of frataxin [113]. Therefore, a deeper understanding of how miR-886-3p regulates frataxin is essential for developing new treatments for FRDA.

Therapeutic Potential of MicroRNAs

RNA interference (RNAi) is a powerful tool for gene silencing and has the potential to become a major class of therapeutic drugs [114]. Its mechanism is naturally mirrored by cells that use microRNAs (miRNAs) to regulate their genes. miRNAs are now recognised for their role in neurodegenerative diseases, with therapeutic strategies under investigation including miRNA mimics and anti-miRNAs [115]. miRNA mimics are synthetic molecules resembling natural miRNA precursors and are designed to suppress specific proteins implicated in disease diseases. However, challenges such as off-target effects, the risk of overloading the RNAi machinery, and the unknown *in vivo* half-lives of these mimics exist. To maintain persistent effects, repeated administration or viral expression may be necessary. Delivery to target cells, particularly across the blood brain barrier, remains a hurdle. A novel delivery method involves exosomes engineered to carry siRNA to the brain, which reduces gene expression in mice without triggering immune responses [116].

The second therapeutic avenue involves anti-miRNAs, designed to block overexpressed miRNAs. Antagomirs, cholesterol-conjugated RNA molecules, have shown promise in reducing miRNA levels in multiple organs when administered intravenously, although they do not cross the blood brain barrier. Locked nucleic acids (LNAs) offer an alternative, with modifications that enhance specificity and reduce dosage needs, and they have shown durable effects. Strategies also include antagomiRzymes that cleave target miRNAs and miRNA sponges, which sequester miRNAs to inhibit their function [117,118].

While promising, these strategies require further research to optimise delivery, efficacy, and safety before they can be considered for human therapy.

Conclusion

MicroRNA has emerged as a significant player in the pathogenesis of neurodegenerative diseases, providing a fresh perspective for uncovering previously unknown molecular mechanisms underlying these conditions. This insight is not only pivotal for understanding the initiation and progression of these diseases at the molecular and cellular level but also opens up possibilities for employing miRNA as a potential therapeutic tool. Specifically, the role of miRNA networks in sporadic neurodegenerative

diseases is a crucial area of study. Gaining a comprehensive understanding of this aspect can pave the way for strategies aimed at modulating miRNA activities, ultimately leading to potential disease-modifying treatments for these otherwise incurable disorders.

Neurodegenerative diseases are often complex and multigenic, requiring therapies that target multiple genes or cellular pathways. The ability of a single miRNA molecule to inhibit the expression of numerous target genes, particularly those functioning in concert within a cellular pathway, suggests that it may be possible to alter an entire disease phenotype by modulating just one miRNA molecule. This approach contrasts with the use of single small interfering RNA (siRNA), which typically targets only one gene and may be less effective in addressing complex diseases.

The rapid progress in the field of miRNA research in recent years, along with the expected acceleration in our understanding, is set to offer new opportunities for addressing the fundamental causes of incurable neurodegenerative diseases. The potential to manipulate miRNA for therapeutic purposes holds great promise, representing a significant advance in the fight against these challenging disorders.

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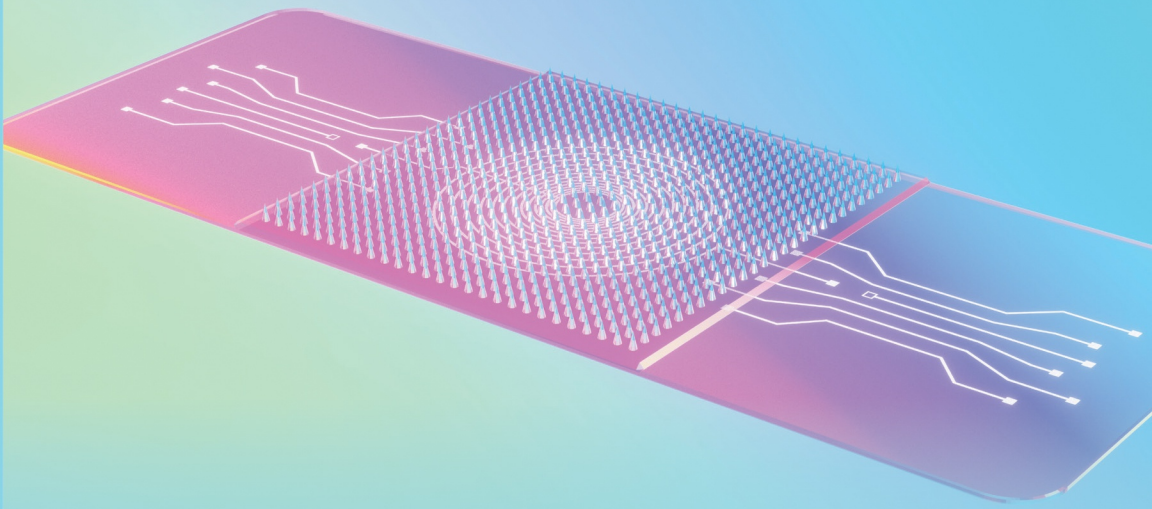

Design and Applications of Microneedles in Drug Delivery and Therapeutics

Edited by

Prashant Kesharwani

DESIGN AND APPLICATIONS OF MICRONEEDLES IN DRUG DELIVERY AND THERAPEUTICS

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DESIGN AND APPLICATIONS OF MICRONEEDLES IN DRUG DELIVERY AND THERAPEUTICS

Edited by

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Dedication

I humbly dedicate this book to my beloved parents, Mr. Hariom Kesharwani and Mrs. Anguri Kesharwani, whose unwavering support and guidance have been the cornerstone of my journey. Additionally, I extend heartfelt gratitude to my cherished wife, Garima Kesharwani, and our dear children, Yashsavi and Vidvaan, whose love and sacrifices have inspired me every step of the way. I am also deeply grateful to my sister, Dr. Punam Kesharwani, and Jiia Ji, Mr. Pankaj Gupta, as well as my brother, Er. Pankaj Kesharwani and his wife Shweta, along with my nephews, Adhyayan and Vishwaraj, and niece, Prisha, for their unwavering belief in me and their constant presence in my life.

Prashant Kesharwani

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Prashant Kesharwani

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CHAPTER 1

Introduction of anatomy of the skin and microneedles as a smart carrier for drug delivery

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1 Introduction

The biggest and most important structure in the body is the skin [1]. The epidermis of a 70-kg individual weighs over 5 kg and has an entire area approaching 2 m^2 [2]. Human skin comprises of a stratified, cellular epidermis as well as a connective tissue-based dermis [3]. A covering of subcutaneous fat lies beneath the dermis and is differentiated from the remaining tissue of the human body through a layer (vestigial) of muscle with striated fibers [4].

Keratinocytes comprise the majority of the epidermis, which is typically 0.05–0.1 mm thick. It is formed by the division of basal layer cells that form the spinous layer [5]. This layer is composed of cells that migrate outward and differentiate progressively to form the granular layer along with the stratum corneum [6].

1.1 Anatomy of skin

The progression of cells through the innermost layer to the skin's surface normally takes approximately 30 days; however, it progresses faster in medical conditions such as psoriasis [7]. A cytoskeleton composed of intermediate keratin filaments gives keratinocytes their "brick-like" morphology [8]. The keratinocytes become flattened as the epidermis differentiates; this process involves filaggrin, a structural protein that is a component of granules of keratohyalin that aggregates filaments [9]. Interestingly, keratin and filaggrin constitute 80%–90% of the epidermis layer covering. The stratum corneum is the outermost covering of the epidermis, where cells (now termed

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corneocytes) lack nuclei as well as cytoplasmic organelles [10]. The corneocyte constitutes a highly insoluble, cornified surrounding underneath the plasma membrane, developed through the a cross-link of easily soluble protein precursors, including involucrin and loricrin, with the latter resulting in 70%–85% of the mass of the cornified cell envelope; it additionally consists of several lipids (fatty acids, sterols, or ceramides) distributed from lamellar bodies on the top, residing in the epidermis, which contains melanocytes, Langerhans' cells, and Merkel cells [11,12]. Melanocytes are dendritic cells, which distribute melanin pigment packages in the melanosomes to neighboring keratinocytes to impart color to the epidermis [13].

The overall number of melanocytes in the white and black epidermis differs barely. Rather, the composition of the melanin as well as the size of the melanosomes is responsible for the various appearances [14]. In addition to being dendritic in nature, Langerhans cells possess a mesenchymal origin along with emanate from bone marrow [15]. Langerhans cells are cell types that process antigens encountered by the epidermis and deliver them to local lymph nodes; they therefore play a vital part in the adaptive immune response in the skin. Merkel cells likely originate from keratinocytes. They serve as mechanosensory receptors that detect contact [16].

There are pilosebaceous follicles in addition to sweat glands in human epidermis. The hair follicles consist of epithelial pockets that are continuous with the epidermis of the skin's surface but also encase a tiny papilla of superficial layer at their base [17]. The arrector pili is a cluster of smooth muscles that descends at an angle from the outermost layer of the dermis to an opening inside the follicle wall [18]. In addition to the point of attachment, there are sebaceous glands, which protrude into the pilary canal that is holocrine. In certain locations, including the axillae, follicles might be accompanied by apocrine glands [4]. The eccrine perspiration glands also stem from the epidermal layer and connect onto the skin surface [19]. The epidermis and dermis are connected by a complex network of proteins as well as glycoproteins that extend from within basal [20]. Along with adhesion, dermatological-epidermal junction constituents also play a role in epithelial-mesenchymal signaling processes including cell migration (for instance, during the healing of wounds) [21]. Throughout a membrane in the basement zone that is less than 200 m in size, interactions between more than 30 different macromolecules (integrins, laminins, and collagens) can be seen [22].

The dermis is a ground substance or supporting matrix in which proteins and polysaccharides are connected to create macromolecules with an exceptional ability for holding water. The dermis can range in thickness from less

than 0.5 mm up to greater than 5 mm [23]. The two main categories of protein fiber are elastic tissue and collagen. The primary matrix's extracellular protein, collagen, makes up 80%–85% of the dermis' dry matter [24].

There are 29 different types of collagen that have been identified in vertebrate tissue (shown by Roman numerals from 1 to XXIX in the sequence of their discovery), at least 12 of which are represented in the skin [25]. The primary basement membrane's collagen (which is primarily at the dermal-epidermal junction and surrounding dermal blood vessels, nerves, and appendages) is type IV collagen, whereas the predominant interstitial dermal collagens in order are types I and III [26]. Although fibrils and fibers are formed from the polymerization of triple-helical collagen monomers, they are sustained by the intricate building up of intra- as well as intermolecular cross-link (Fig. 1) [27].

The skin gets its tensile strength from collagen fibers, which are incredibly resilient. Elastic microfibrils, which envelop the elastin in addition, can extend all throughout the dermal layer in a web-like pattern to the interface between the dermis and the epidermis, are made up of a number of proteins, including fibrillin. Along with collagen, it is composed of noncollagenous glycoproteins such integrins, fibronectins, and fibulins [28]. These elements of the extracellular matrix promote cell adhesion and motility. Glycosaminoglycan/proteoglycan macromolecules constitute the ground substance that lies between the dermal collagen and elastic tissue [29]. These comprise only 0.1%–0.3% of the dermis' total dry weight but are essential for maintaining hydration because of hyaluronic acid's strong capacity to bind water. Water makes up approximately 60% of the dermis' overall weight [30].

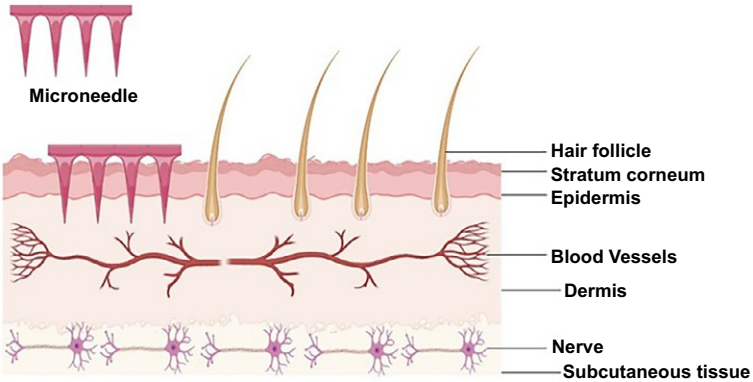


Fig. 1 Structure of the skin and its associated appendages.

1.2 Functions of skin

Providing a mechanical defense against the elements of the external realm is one of the skin's primary functions [31]. In addition to preventing water loss from the skin, the stratum corneum and the naturally occurring antimicrobials (defensins and cathelicidins) generated by keratinocytes offer an innate immunological defense against bacteria, viruses, and fungi [32]. A network of around 2109 Langerhans cells, which act as sentinel cells and primarily monitor the epidermis environment and trigger an immune response in response to microbial threats, is also present in the epidermis [33]. They may also play a role in the tolerance of immune cells in the skin. Melanin, which is mostly present in the basal keratin cells, also offers some defense against UV radiation-induced DNA damage [34].

Thermoregulation is one of the skin's key functions. Heat loss is controlled by vasodilatation or constriction of the body's blood vessels within the deeper or superficial plexuses. All skin sites have eccrine sweat glands, which range in density from 100 to 600/cm^2 [35]. They help regulate body temperature and generate about 1 L of sweat per hour throughout light exercise [36]. Pheromones, which are produced by apocrine sweat glands, are a component of body odor. Sebum, which is released by the sebaceous glands, lubricates and waterproofs the skin. Along with acting as a barrier and a calorie store, subcutaneous fat plays crucial roles in protecting against injury [37]. Approximately 80% of the body's total fat in nonobese individuals is located in the subcutaneous tissue [38]. Leptin is a hormone that is released from fat and affects the hypothalamus to control appetite and energy metabolism. Additionally, fat cells alter the tissue and engage in phagocytosis. The skin plays a crucial role in the synthesis of numerous metabolic products, including vitamin D [39].

1.3 Skin development

The prospective epidermis, which develops from a surface region of the embryonic gastrula, and the potential mesoderm, which originates in the proximity to the innermost layer of the epidermis during gastrulation are juxtaposed to form the skin [40]. In addition to producing the dermis, the mesoderm is crucial for promoting differentiation of epidermal structures such as the hair follicle. The neural crest is the source of the melanocytes [41].

After gastrulation, the embryo's surface has only one covering layer of neuroectoderm; relying on the chemical signals it receives, this layer develops into either the skin epithelium or the nervous system [42].

Wnt signaling activation prevents the ectoderm from responding to fibroblast growth factors (FGFs). The cells exhibit bone morphogenic proteins (BMPs) while growing into an epidermal lineage in the absence of FGFs. Contrarily, neuronal destinies are promoted by a lack of Wnt signaling [43].

The periderm, a distinct layer that exists solely in mammals, covers only one layer of multipotent epithelial cells that make up the embryonic epidermis [44]. The periderm protects the developing skin in various ways and facilitates material exchange through the amniotic fluid during pregnancy [45]. The embryonic dermis initially has a high cell density, and between 6 and 14 weeks, three distinct cell types—stellate cells, phagocytic macrophages, and granule secretory cells—melanoblasts or mast cells—are present [46]. Fibroblasts are numerous and active from weeks 14 to 21, and perineural cells, pericytes, melanoblasts, Merkel cells, and mast cells can also be distinguished [47]. The different embryonic time points at which the structural elements of the skin that may be observed postnatally first foster include follicles for hair and nails in 9 weeks, sweat glands in 9 weeks for the soles and palms, and 15 weeks for other sites, as well as sebaceous glands, which produce sebum in 15 weeks [48]. By week 6, touch pads are noticeable on the tips of the fingers and toes, and by week 15, they have developed to their fullest extent, then they flatten and lose their definition, and the pattern of dermatoglyphics that replaces them is determined by these locations [49]. Fig. 2 illustrates the major components of the skin as well as the numerous drug delivery systems that can be used to administer drugs via these systems through the layers of the skin.

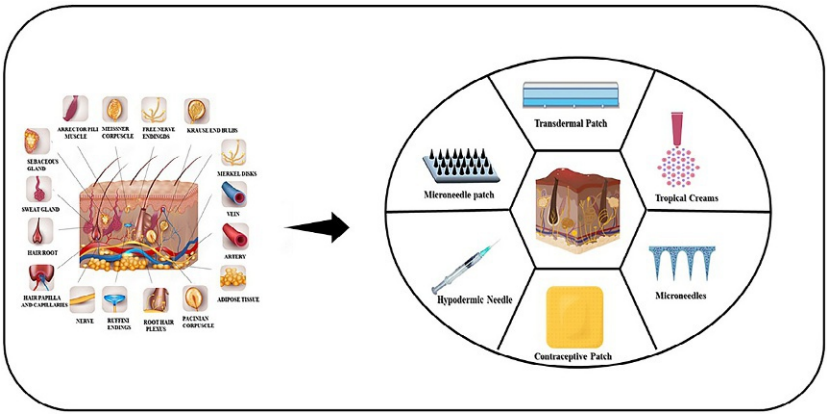


Fig. 2 Shows skin components and drug delivery mechanisms.

2 Microneedles as a smart carrier for drug delivery

Researchers explored microneedles (MNs) as a way to overcome the limitations of more traditional methods of drug delivery and expand the potential applications of transdermal drug delivery [50,51]. Microneedle device comprises of microscopic needles arrayed on a tiny square. The microneedle drug delivery device was designed as a compromise between the hypodermic needle and the transdermal patch. Microneedles are a refined technology that has allowed researchers to penetrate the stratum corneum with hydrophilic high-molecular-weight substances [52,53]. Using a microneedle device for drug delivery increases the number of drug molecules that penetrate the skin by allowing them to pass through the stratum corneum [54]. The advantages of this technology include a faster onset of action, more patient compliance, the opportunity for patients to self-administer the medicine, and enhanced permeability and efficacy. Microneedles have numerous therapeutic benefits, including the fact that their outcomes are highly accurate and reproducible, with little to no variation between subjects. The major reason for developing this technique is to increase permeability through the stratum corneum through the development of larger transport pathways of micron size, which is larger than molecular dimensions but smaller than holes by hypodermic needles [55,56]. Only 10%–20% of the medication content in the cream is reportedly being absorbed by the skin. Transdermal patches have lower bioavailability since the drug must first cross the stratum corneum [57]. Transdermal patches with added permeability enhancers can increase drug absorption to a limited extent. The hypodermic needle enters the dermis to a depth where pain receptors are located [58]. As a result, it can deliver 90%–100% of the drug load, but the pain is so severe that patients rarely use it. To deliver 100% of the loaded drug easily, microneedle patches bypass the stratum corneum barrier and administer the drug directly into the epidermis or upper dermis layer [59]. Yang, in 2023 for the extended release of rhGH, developed silk protein-based actively separated microneedle patch (PAA/$NaHCO_3$-Silk MN). Silk protein, as a protein-friendly carrier material, might be obtained in mild full-water conditions to ensure the efficacy of recombinant human growth hormone (rhGH). Active separation is done by absorbing the interstitial fluid (ISF) to activate HCO_3 in the active backing layer to produce carbon dioxide gas (CO_2) after the PAA/$NaHCO_3$-Silk MN patch has been manually applied to the skin for 1 min. Compared with daily subcutaneous (S.C.) injections of rhGH, the MN patch produced similar results in boosting height and weight in rats and was well tolerated. The potential for easy self-administration, and the

fact that the PAA/$NaHCO_3$-Silk MN patch doesn't need to be kept in a cold chain, is further advantageous economically [60].

2.1 Mechanism of drug delivery

The diffusion method is also used in topical administration of drugs. The skin is momentarily pierced by the microneedle drug delivery device [61]. Hundreds of microneedles are arrayed on a tiny patch (the same size as a standard transdermal patch) to produce a microneedle device that can provide the necessary dose of drugs for a therapeutic effect. It gets beyond the protective layer by penetrating the stratum corneum. The drug is topically applied to the epidermis or higher dermis, where it is absorbed into the bloodstream and carried to the area of the body where it will have the most therapeutic effect [62].

2.2 Microneedle's dimension

Depending on the microneedle's design and the substance it is formed from, it can be fabricated in a range of sizes. Needles of up to 1500 m in length are enough for medication delivery to the epidermis, given that its thickness might reach up to 1500 m [63]. Larger and wider needles are able to penetrate deeper layers of the skin, where they might cause more nerve damage and discomfort. They normally have dimensions of 150–1500 m in length, 50–250 m in width, and 1–25 m in tip thickness. Needles are limited to a diameter of a few microns so as to facilitate the development of a transport pathway on the microscale [64].

2.3 Fabrication material

2.3.1 Silicon

Back in the 1990s, the first silicon microneedle was fabricated. Silicon has a crystalline structure and exhibits anisotropy. The crystal lattice orientation affects its characteristics, with elastic moduli ranging from 50 to 180 GPa. Because of its malleability, it can be used to obtain needles in a wide range of sizes and configurations [65]. It's a versatile substance thanks to its appealing physical qualities. Precision manufacturing and batch production are both possible with silicon substrates. However, its usage in microneedles is restricted by its high cost and the complexity of its manufacture method. Silicon is brittle, so if anything breaks off and gets stuck under the skin, it might potentially cause health problems [66].

2.3.2 Metal

The most common materials are titanium and stainless steel. Additional materials include nickel, palladium, and palladium-cobalt alloys. They're biocompatible and have excellent mechanical qualities. Metals are more robust than silicon and can withstand being used as microneedles without breaking. Stainless steel was the first material utilized in the microneedle manufacturing process. Titanium can be used as a substitute for stainless steel [67,68].

2.3.3 Ceramic

Alumina (Al_2O_3) is popular due to its durability against chemicals. The highly energizing ionic and covalent interactions between the Al and O atoms allow for the formation of a stable oxide. Gypsum ($CaSO_4\ 0.2H_2O$), a calcium sulfate dihydrate, and brushite ($CaHPO_4.2H_2O$), a calcium phosphate dihydrate, are two other common ceramics [69]. Ormocer, an organically modified ceramic, has been used extensively in recent years. It is a copolymer that is cross-linked in all three dimensions. Utilizing a variety of organic components during the polymerization process allows for the formation of a polymer with tailored properties [70]. Micromolding is the primary method of production. A micromold is used to cast ceramic slurry. Micromolding techniques provide the potential for scale-up while still being cost-effective [71].

2.3.4 Silica glass

Glass allows for the microscale production of a wide variety of shapes. Silica glass is a brittle yet scientifically inert material. Glass constructed from silica and boron trioxide, known as borosilicate glass, has more elasticity [57]. Being predominantly hand-made makes them less productive in terms of time. Nowadays, MNs made of glass are only utilized in research labs.

2.3.5 Carbohydrate

Maltose is a popular sugar substitute. In addition to glucose, a variety of other sugars and polysaccharides can be utilized. Silicon or metal molds are used to shape slurries of carbohydrate [72]. Microneedles are produced by casting a drug-loaded carbohydrate mixture into molds. Drug release beneath the skin can be controlled by the pace at which carbohydrates dissolve. Carbohydrates are inexpensive and nonhazardous to human health, but their decomposition at high temperatures hampers the manufacturing process [73].

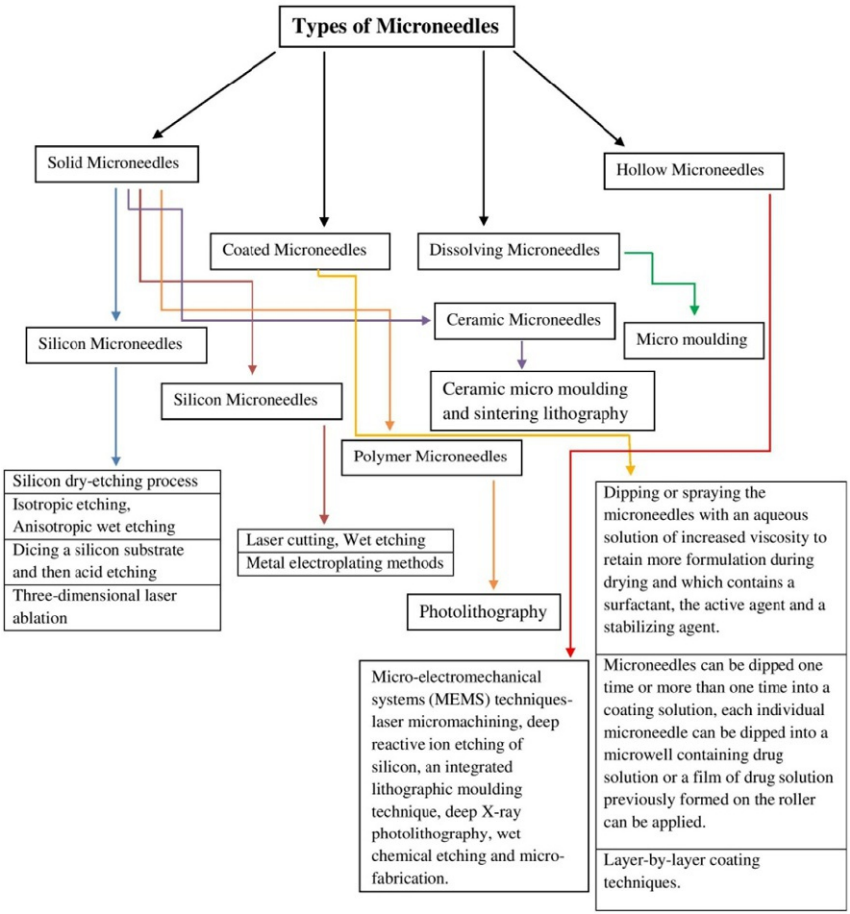
2.3.6 Polymers

Poly (methyl methacrylate) (PMMA), poly (lactic-*co*-glycolic acid) (PLGA), polyglycolic acid (PGA), poly (carbonate), and many more are all examples

of polymers [74]. It has been claimed that SU-8 photoresist [75], poly (vinyl-pyrrolidone) (PVP) [76], poly (vinyl alcohol) [77], polystyrene [78], poly (methyl vinyl ether-comaleic anhydride) [79], and cyclic-olefin copolymer can all be used to develop microneedles [80]. These polymers are commonly used to fabricate hydrogel-forming microneedle arrays that can dissolve or biodegrade. The polymers can be used to produce microneedles that are tougher than glass or ceramic but not as strong as metal [81].

2.4 Fabrication technique

When considering a fabrication or manufacturing method for microneedles, it is important to consider the microneedle's type, geometry, and material [82–84].



2.5 Types of microneedles

Solid, coated, dissolving, hollow, and hydrogel microneedles have all been produced and studied for their potential use in drug delivery as shown in Fig. 3 [85]. Microneedles come in a wide variety, with each offering its own unique method for injecting drugs beneath the skin's surface [86]. Some are used solely to form pores in the stratum corneum, while others are precoated with the drug solution on their surface, while others are soluble or prefilled with the drug [52].

2.5.1 Solid microneedles

When used as a pretreatment, solid microneedles generate pores in the skin. When a drug patch is applied, the pointed needles puncture the skin, creating micron-sized channels through which the drug can enter the skin and have a more profound impact. To produce a systemic impact, the drug must first be absorbed by the capillaries. A local effect is also possible with this. The drug is gradually diffused across the skin layers using solid microneedles [87].

2.5.2 Coated microneedles

The drug solution or dispersion layer surrounds the microneedles. Subsequently, drug dissolution from the layer occurs, and the drug is rapidly administered. The amount of drug that may be added onto the needle is determined by its diameter and coating thickness [88].

2.5.3 Dissolving microneedles

Biodegradable polymers are used to develop dissolving microneedles by enclosing the medication within the polymer. When a microneedle is inserted into the skin, the medicament is dissolved and then released. Since the microneedle need not be withdrawn from the body upon insertion, the procedure can be completed in a single step. The degradation of the polymer under the skin regulates the release of the drug. One of the finest options for long-term therapy with increased patient compliance is the use of a polymer that is bioacceptable and dissolves inside the skin. The development of dissolving microneedles highlights challenges related to the effective distribution of needle drugs. Because of this, blending the polymer and drugs together is an essential process [89].

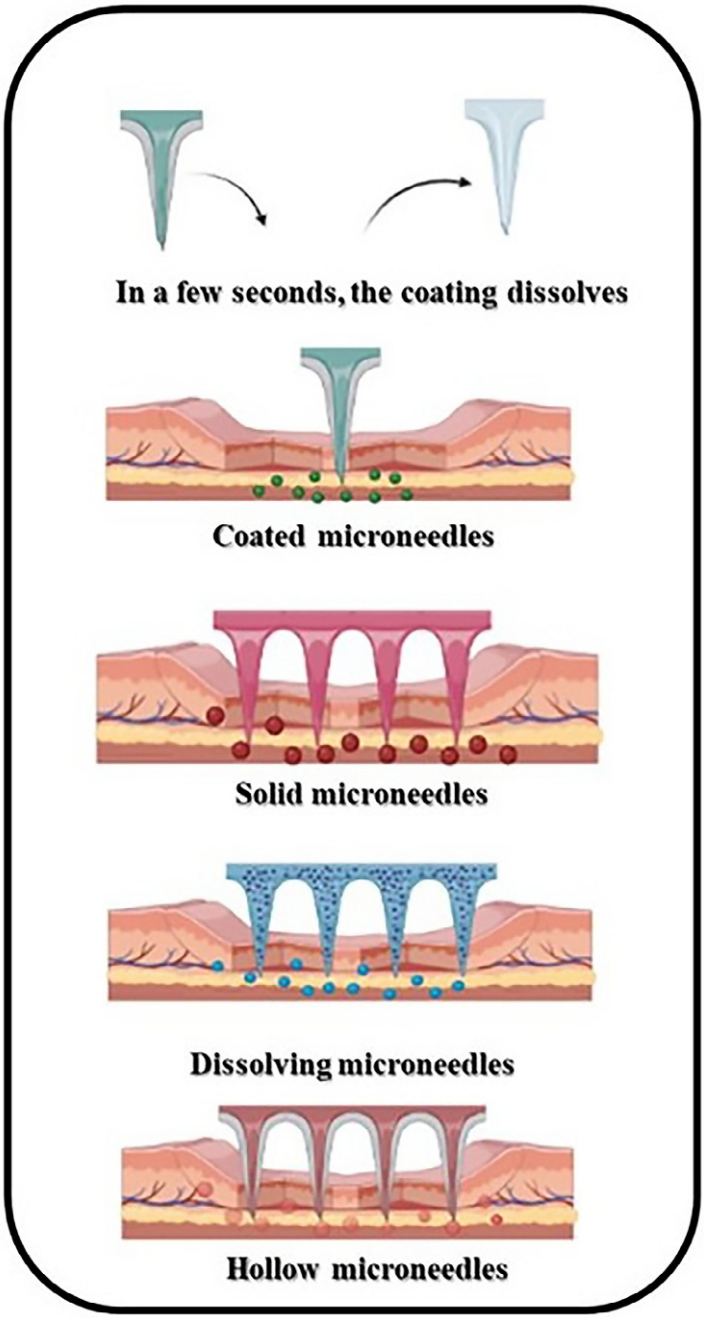


Fig. 3 Different types of microneedles.

2.5.4 Hollow microneedles

Microneedles that are hollow can be preloaded with a drug solution or dispersion. When a drug delivery system is inserted into the skin, it deposits the medicinal product in the epidermis or the top layer of dermis. Typically, its large-molecular-weight proteins, vaccines, and oligonucleotides are all examples of such molecules. If the medicament is to be administered as a rapid bolus injection, the flow rate and release pressure can be modified. These microneedles can inject a sizable quantity of drugs because they have a larger volume inside than conventional needles. Here, it is crucial to keep the flow rate steady. Increasing the microneedle's bore can boost its flow rate, but at the expense of its durability and precision. A metal coating can be applied to the microneedle to boost its strength; however, this can increase the sharpness of the needles [90].

2.5.5 Hydrogel-forming microneedles

This microneedle is a very new invention. Microneedles are produced using superswelling polymers. Polymers make up the hydrophilic structure, which allows for significant adsorption of water into their cellular polymer network in three dimensions. When inserted under the skin, these polymers expand because of the presence of interstitial fluid. This results in the development of capillary-like channels connecting the drug patch and the body's circulatory system. These microneedles are used just to break down the skin's protective barrier prior to needling. When expanded, they behave like a regulator membrane. Size and form can be altered easily. These microneedles are distinct in that they are simple to sterilize and may be removed from the skin without damaging it [91].

2.6 Methods of delivery

Delivery of drug into the epidermis can be accomplished in a number of ways. Microneedles can be used to poke holes in the skin, and then the drug-containing patch can be applied over the holes [92]. This makes it possible for drugs to be absorbed directly into the skin. Better results can be achieved by using an electric field. The second method involves applying a coating layer containing drug to the microneedle's surface [93]. Drugs are dissolved in the skin once the coated microneedles are implanted. The third method involves immersing the microneedles into the drug solution and then scraping them into the skin [88,94]. The drug is absorbed by the wounds. The medicament might also be combined with a biodegradable

polymer before being used to produce the microneedles. The drug solution can be injected directly into the body via a hollow microneedle [95].

2.7 Evaluation of microneedles

2.7.1 Characterization methods

The drugs can be encapsulated (in liposomes, nanoparticles, or nanoliposomes) or injected onto or into the microneedles in the form of a suspension/dispersion. The polymer solution can be used to coat the drug or construct a patch to deliver the medicament. Depending on the formulation utilized in the microneedles, different physicochemical characterizations, such as particle size, polydispersity index, viscosity, and zeta potential, can be assessed for the loaded drugs [96]. Tests are carried out on a patch that is applied following pretreatment to determine its drug release, adhesion, and penetration capabilities. Dynamic light scattering, X-ray scattering, and transmission electron microscopy can be used to determine the size, internal structure, and crystallinity of the liposomes or nanocarriers, respectively [97]. Drug dispersion and microneedle stability investigations can be conducted in environments that mimic those found in living organisms, such as those found in cultured cells or animal tissues. Biocompatibility, in-vitro release, solubility, and other evaluation are also conducted on developed microneedles [98,99].

2.7.2 Dimensional evaluation

The needle's geometry is assessed by a number of techniques, and dimensions such as the needle's tip radius, length, and height are measured. Optical and electrical microscopies are the most prevalent techniques [100]. Examining a 3D image helps with quality control by providing a clearer picture of the needle's geometry. This has been accomplished with the use of a confocal laser microscope and a scanning electron microscope (SEM). Scanning electron microscopy (SEM) uses a focused stream of electrons that interact with the atoms in a sample during scanning to provide an image of the sample's surface topography and composition. High-resolution images are captured by confocal laser microscopy [101,102].

2.7.3 Mechanical properties or insertion forces

A microneedle needs to be both sharp and thin to easily penetrate the skin, yet sturdy enough to prevent injury once it's inside. Safe and effective microneedle design is dependent on two factors: the insertion force and the force at which the microneedle breaks substantially [100]. The "safety

factor" refers to the proportion between these two forces. It's ideal if the ratio is quite high.

2.7.4 In-vitro skin permeation studies

Using a diffusion cell apparatus, one can determine the amount of drug that penetrates through the skin. The experiment is primarily based on pig ear skin that is situated between a receptor compartment and a donor compartment. A comparison was made between the cumulative permeation profiles of skin that had been treated with microneedles and skin that had not been treated [103].

2.7.5 In-vivo animal model studies

Rats bare of body hair might be used in the study. Humane sedation techniques must be used on the animal. One sign that is measured both before and after microneedling is the transepidermal water loss. A Delfin Vapometer was utilized in the process of determining this value [104].

3 Applications of microneedles

Recent advances in production methods have allowed MNs to find widespread use in areas that are as diverse as immunobiology, illness detection, and the beauty industry. The first MNs found their niche as an invaluable tool in the study of experimental embryology, tissue culture, and electron microscopy in the laboratory. In the 1980s, MNs saw a surge in popularity for use in the transport of proteins, DNA, and other biomolecules in the context of laboratory cell engineering. Transdermal MN administration was patented in the United States at the same time as the initial proposal for MNs in 1976 [105]. Although MNs were first proposed in the 1970s, it took nearly two decades for the technology to be developed to mass produce micron-sized needles [106]. Many researchers in the medical field are interested in MNs. There has been constant research and innovation into the production and layout of MNs, and they are now in the clinical trial phase.

3.1 Treatment of osteoarthritis and rheumatoid arthritis

More than 250 million people around the world are living with the debilitating effects of osteoarthritis (OA), making it the most prevalent form of joint illness [107]. The disease treatment and symptomatic treatment of osteoarthritis are in high demand because it is the most common form of arthritis and a leading cause of disability. However, there is still a lack of effective

treatment options for OA, and the available options are limited. MN percutaneous administration is preferable to parenteral administration because it is less invasive [108]. On the other hand, the digestive system and the liver can degrade enzymes, but this is avoided with percutaneous administration [109]. Potential benefits of MNs for OA treatment include avoiding liver metabolism, lowering the risk of severe gastrointestinal adverse reactions to oral drugs, achieving long-term and sustained drug release, and quickly and easily preventing drug delivery in the event of toxicity.

Methotrexate, artemether, alkaloids, capsaicin, neurotoxic, triptolide, paeoniflorin, sinomenine, and Etanercept (EN), among others, have all been coupled with MN technology to treat rheumatoid arthritis. As a result, the transdermal distribution of MN is a potentially effective treatment for arthritis-related disorders. Minimally invasive administration of drug is utilized to regulate drug release, lessen dosage, and reduce or eliminate the potential for unwanted side effects [106,110]. The constraint of drug solubility was overcome by using a nanolipid carrier as the drug carrier and encapsulating the drug with the carrier until it was distributed by soluble MNs. The ability to transdermally transport most drugs is constrained by the stratum corneum (SC) barrier [111]. In consideration of this, researchers designed tetramethylpyrazine-loaded dissolving microneedle patches (TMP-DMNPs) and assessed its antirheumatoid arthritis effect. The mechanical strength of the cone-shaped dissolving microneedle patch was exceptional, and the needles were all present and properly organized. When applied to the skin, it might easily break through the protective barrier of the stratum corneum. The transdermal penetration of TMP was greatly increased by DMNPs (7 h) in an in vitro transdermal experiment compared with TMP-cream (5 h). In vivo dissolution studies showed that the needles dissolved within 18 min, and the treated skin was back to normal after 3 h. Human rheumatoid arthritis fibroblast synovial cells responded favorably to the excipients and blank DMNP in terms of safety and biocompatibility. The animal model was developed for the purpose of evaluating the therapeutic effects. Dissolving microneedles dramatically improved paw condition, decreased serum concentrations of proinflammatory cytokines, and suppressed synovial tissue damage in rats with AIA (adjuvant-induced arthritis) [112]. In 2021, a group led by Ping Zhou developed a liposome-loaded dissolving microneedle (DMN) technology that successfully delivers poorly water-soluble triptolide (TP) to alleviate osteoarthritis (OA) symptoms. Triptolide liposome (TP-Lipo) with an entrapment effectiveness of 90.25% was developed and injected into DMNs to deliver TP. After being

concentrated in an ultrafiltration tube and combined with a hyaluronic acid solution to make DMNs, TP-Lipo-loaded DMNs (TP-Lipo@DMNs) were able to insert themselves into the dermal layer of rat skin and penetrate approximately 200 m. When compared with intraarticular injection, TP-Lipo@DMNs had a prolonged effect in the drug distribution in vivo. TP-Lipo@DMNs considerably decreased knee joint swelling and the level of inflammatory cytokines (TNF-α, IL-1β, IL-6), according to in vivo pharmacodynamic studies. TP-Lipo@DMNs were found to successfully mitigate OA symptoms and decrease cartilage breakdown, as measured by micro-CT and histological analysis [113].

Polydimethylsiloxane micromolds were used to develop a microneedle patch made of low-molecular-weight polyvinyl alcohol and polyvinylpyrrolidone. Maximum needle strength was accomplished by adjusting the solid concentration of the matrix solution and the polyvinyl alcohol to polyvinyl pyrrolidone ratio. Dissolution, drug release, stability, ex vivo skin permeation/deposition, histopathology, and in vivo pharmacodynamics were all tested on the optimized batch. Maximum axial needle fracture force (0.9 N) acceptable for penetrating the skin was demonstrated by the patch comprising a 9:1 polyvinyl alcohol to polyvinylpyrrolidone ratio with 50% solid content. Using dissolution controlled kinetics, the optimum batch was found to dissolve rapidly and release nearly 100% medication in 60 min. The formulation revealed a 2.58-fold increase in permeability compared with plain drug solution, significant drug deposition within skin (63.37%), and better transdermal flux (1.60 $g/cm^2/h$). When compared with the already marketed and approved oral tablet, the new formulation demonstrated similar antiinflammatory efficacy in rats. The stability and histopathology tests proved that the developed formulation was safe to use [114].

3.2 Treatment of dermatology dermatosis

The skin is the body's first line of defense against the environment, and skin-related diseases are one of the most active areas of study for MNs today. Mesh's ability to prevent infection and encourage tissue remodeling after skin injury is highly beneficial [115]. Common patches have a weak healing effect and limited medical applications due to their basic microstructure and sluggish medication administration. Because of their porous microstructures, MNs could be used to encapsulate skin growth factor or medicines for prolonged release or controlled release using antibacterial multifunctional polymer materials [106]. Dissolvable microneedle arrays (MNAs) have

been evaluated for their potential to be used for intradermal localized delivery of anti-TNF-α Ab. Micromilling/spin-casting was used to obtain carboxymethylcellulose (CMC) MNAs with obelisk-shaped microneedles that contain the antibody cargo in the needle tips. The conformational dependence of TNF-α binding activity was preserved when anti-TNF-α Ab was incorporated into MNAs utilizing this room-temperature manufacturing technique. The binding affinity of MNA-integrated anti-TNF-α Ab was similar to that of fresh unintegrated Ab (9.33 ± 3.18 pM on average), although somewhat decreased (50.6 ± 5.84 pM on average). Furthermore, these MNAs transported anti-TNF-α antibodies to the dermis of human skin with a release profile suitable for therapeutic use. We used MNAs with anti-TNF-α Ab to test its efficacy in treating psoriasiform lesions on mice's skin. Epidermal thickness and IL-1β expression, two critical biomarkers of psoriasiform inflammation, were both reduced by treatment with MNA anti-TNF-α Ab. Together, these findings provide credence to the ongoing search of MNA-mediated antibody delivery for clinical applications, since they show that MNAs can efficiently and biologically effectively carry anti-TNF-α Ab to the intradermal microenvironment of the skin in mice and humans [116]. In 2022, Men and fellow workers synthesized tacrolimus (TAC) nanocrystals (NCs) by dispersing TAC into a sodium hyaluronate-based microneedle patch (MNP), and they assessed the MNP's therapeutic efficacy. TAC NCs had an average particle size of 259.6 ± 2.3 nm. Microneedles with a mechanical strength of 0.41 ± 0.06 N/needle successfully penetrated psoriatic skin. After 10 min, the microneedles were removed from the substrate and inserted 258.8 ± 14.4 μm into the psoriasis-affected skin. The MNP (8.40 ± 0.33 μg/cm^2) had six times the intradermal retention of the commercial ointment (1.40 ± 0.12 μg/cm^2) compared with the control group. The phenotypic and histological aspects of psoriatic skin were improved, and the levels of TNF-α, IL-17A, and IL-23 were reduced, according to the results of pharmacodynamic examinations [117].

3.3 Delivery of vaccine

Vaccine delivery with MNs is a more developed field because it has been researched continuously. In particular, MN vaccine can be used for the packaging or encapsulation of DNA vaccine, subunit antigen, or inactivated or live virus vaccination, and it can be maintained at room temperature and transported in solid form [118,119]. Also, the elimination of the need for a

cold chain in the storage and transportation of reagents and associated reduction in sharp medical waste makes this a painless, noninvasive, and incredibly convenient method of managing and transmitting these substances. Additionally, in places without adequate medical facilities or management, such as rural areas or developing nations, it can lessen the damage caused by and spread of infectious blood-borne diseases [120]. In 2023, scientists developed a dissolving microneedle patch (dMNP) to deliver 5, 10, and 20 μg of adjuvant-free monovalent vaccine (AFV) hepatitis B surface antigen (HBsAg) via intramuscular (IM) injection and compared its immunogenicity with vaccination with 10 μg of standard monovalent HBsAg delivered via IM injection, either as AFV or as aluminum-adjuvanted vaccine (AAV). Mice were vaccinated three times (0, 3, and 9 weeks), and rhesus macaques were vaccinated 0, 4, and 24 times. Protective anti-HB antibody responses ($\geq$10 mIU/mL) were elicited by vaccination with dMNP in mice and rhesus macaques at all three HBsAg dosages tested. In mice and rhesus macaques, HBsAg administered through dMNP elicited greater anti-HBsAg antibody (anti-HBs) responses than the 10 μg IM AFV, but lesser responses than the 10 μg IM AAV. CD4+ and CD8+ T-cell responses to HBsAg were found in all vaccination groups. Differential gene expression profiles for each vaccine administration group showed that all groups showed activation of the tissue stress, T-cell receptor signaling, and NFκB signaling pathways. These findings indicate that the signaling mechanisms used to trigger innate and adaptive immune responses by HBsAg given by dMNP, IM AFV, and IM AAV are all identical. Researchers also showed that dMNP maintained $67 \pm 6\%$ HBsAg efficacy when stored at room temperature (20°C–25°C) for 6 months [121]. Chen and his coworkers, in 2020, developed F1 antigen-loaded liposomes (F1-liposomes) using the patent formulation of the platform. Further, in vitro testing was conducted with phosphate-buffered saline (PBS) as the solvent to evaluate the F1 protein level of the liposome. Microneedles were used to inject the produced F1-liposomes into Balb/c mice that had functional immune systems. Every 7 days for 45 days, blood was drawn from these immunized mice so that the anti-F1 IgG titer could be measured using enzyme-linked immunosorbent assay (ELISA). In addition, the levels of cytokines interleukin-4 (IL-4), interferon-γ (IFN-γ), and tumor necrosis factor-alpha (TNF-α) were measured 35 days after vaccination with the BIO-Plex proassay to verify the immunological responses. The anti-F1 IgG antibody titer assay, the cytokine response test, and the animal exposure test all showed that F1-liposome-treated mice had the greatest immunity compared with PBS- and F1-Alugel-treated controls [122]. Another study

used SARS-CoV spike glycoprotein (GP) as a model antigen and developed PLGA polymeric microparticles (MPs). Alhydrogel and AddaVaxTM were encapsulated in adjuvant MPs using a similar formulation. The double-emulsion procedure was used to formulate MPs, which were then lyophilized. The range of the MPs' percent recovery yield was between 88% and 91%. Average particle size was measured to be 1.15, 1.19, and 1.05 μm for spike GP MPs, Alhydrogel MPs, and AddaVax MPs, respectively. Researchers discovered that MPs' PDIs fall somewhere between 0.55 and 0.67. The results showed that the zeta potential is between −20 and −25 mV. Protein analysis of Spike GP MPs revealed a total protein content of 88.96 ± 7.58%. When observed in an SEM, both spike GP MPs and AddaVaxTM MPs seem round. Nonspherical shapes were seen in SEM images of Alhydrogel MPs. The release of nitrite, autophagy, and antigen-presenting molecules with their costimulatory molecules revealed the increased immunogenicity of the spike GP MPs + Alhydrogel MPs + AddaVaxTM MPs in vitro [123].

3.4 Treatment of cancer

Minimally invasively and with increased therapeutic efficacy, MNs have been used to transport anticancer drugs (chemotherapy), as well as photodynamic therapy (PDT) and photothermal therapy (PTT) compounds, to skin tumor locations [124]. Cryomicroneedles were used to deliver ovalbumin-pulsed dendritic cells to animals with subcutaneous melanoma tumors. This method resulted in stronger antigen-specific immune responses and slower tumor growth than intravenous or subcutaneous injections of the cells. Cell viability and proliferation capacity may be preserved during delivery via cryomicroneedles [125]. Minimally invasive cell delivery for various cell therapies may be facilitated by the use of biocompatible cryomicroneedles. To better deliver cisplatin to cancerous A-431 epidermoid skin tumors, a unique 3D-printed polymeric microneedle array was developed. Stereolithography (SLA) was used to selectively photopolymerize successive layers of a biocompatible photopolymer resin, and then the microneedles were coated with cisplatin formulations utilizing inkjet dispensing. Microneedle mechanical characteristics and optical coherence were enhanced by optimizing printability via SLA. Microneedles that were 3D-printed and analyzed using tomography penetrated the tissue at a rate of 80%. Rapid cisplatin release rates of 80%–90% within 1 h were found in Franz cell diffusion experiments, and in vivo evaluation in Balb/c nude mice showed adequate

cisplatin permeabilization, leading to high anticancer efficacy and tumor diminution. Tumor growth inhibition was confirmed by histopathology, which revealed well-defined lesions surrounded by thin fibrous capsules and containing necrotic cores. 3D developed microneedles show potential for transdermal in-vivo administration of anticancer drugs [126]. In recent years, photodynamic therapy (PDT) has emerged as a promising option for the treatment of nonmelanoma skin malignancies. Hypericin (Hy) is an effective lipid-soluble photosensitizer that has therapeutic potential in the treatment of cancer. However, its low skin penetration, aggregation in biological systems, and poor water solubility limited its practical application. Researchers provided the first report of Hy being encapsulated in lipid nanocapsules (Hy-LNCs) and then using an array of hollow microneedles (Ho-MNs) from an AdminPenTM and one that was obtained in-house to facilitate efficient intradermal distribution. Ex vivo drug distribution and cellular uptake were measured, along with physicochemical characteristics and photoactivity. Compared with free Hy, the results showed that the particle size of Hy-LNCs was 47.76 ± 0.49 nm, the PDI was 0.12 ± 0.02, the encapsulation efficiency was $99.67\% \pm 0.35$, the drug was deposited sevenfold more deeply into the skin, and the cellular uptake and photocytotoxicity were both significantly increased. Finally, a nude mouse model with transplanted tumors was used to evaluate the therapeutic efficacy of Hy-LNCs in vivo. After being exposed to 595 nm light, the Hy-LNCs that were supplied via Ho-MN showed impressive antitumor destruction (85.84%). This research demonstrated that a minimally invasive, effective, and site-specific strategy to treating nonmelanoma skin malignancies may be formed by the Ho-MN-driven delivery of Hy-LNCs followed by irradiation [127]. The use of chemotherapy drugs applied directly to the skin is a promising new direction in the treatment of skin diseases. However, several different pharmaceutical approaches are required to enable substantial amounts of drugs to enter tumor tissue. In this study, scientists sought to explore a novel treatment strategy for the therapy of melanoma by combining Derma roller microneedles with coloaded liposomes containing doxorubicin HCl (DOX) and celecoxib (CEL). Liposomes/Gels containing both DOX and CEL were obtained and analyzed. The results demonstrated that DOX penetration into the skin was enhanced by roughly twofold following microneedle pretreatment with liposomes gel as compared with passive delivery. Significant growth suppression of B16 cells was seen when either CEL or DOX liposomes were used. In addition, the cytotoxicity of DOX/CEL coloaded liposome was shown to be higher than that of DOX/CEL

solution and single-drug-loaded liposome. Subcutaneous melanoma in female BALB/nude mice was effectively suppressed by transdermal delivery of DOX/CEL coloaded liposomes, and coadministration of DOX/CEL with liposomes was more effective and greatly increased the antitumor effect more than single-drug-loaded liposomes. In addition, using Dermarollers before applying the gel significantly increased the tumor inhibition rate. With its high targeted inhibition efficacy and low side effect profile, microneedle delivery of DOX/CEL coloaded liposomes is a promising technique for treating skin tumors [128].

3.5 Usage in the contraception field

Approaches that give long-term pregnancy protection have gained more interest due to the lower dose frequency and improved patient compliance compared with short-acting contraceptives [129]. The microneedle (MN) patch is a revolutionary transdermal drug delivery device that has found widespread use in a number of biomedical applications, including long-acting contraceptives, owing to its advantageous qualities, such as painless self-administration and the avoidance of biohazardous waste [130]. Unwanted pregnancies can occur when women do not have access to birth control or when they utilize a barrier form of contraception, both of which have low acceptance and a high failure rate [129]. Although sustained-release contraceptive hormone formulations are available, they normally require the assistance of a medical professional for administration. Lee and his team, in 2021, developed a system in which microneedles (MNs) are attached to a porous patch backing, which is strong enough to withstand compression during MN insertion into skin but allows for instantaneous detachment (<1 s) of the MNs upon patch removal under tension from the skin surface. Poly(lactic-*co*-glycolic acid) MNs were formulated to gently release the contraceptive hormone levonorgestrel for up to 1 month after patch application; when removed, the patch produced no biohazardous sharp waste. By providing a straightforward and practical option for self-administered, long-acting contraception, our combination technique of instantaneous MN detachment in the skin and sustained drug release from the MNs could increase access to long-acting contraception [131]. In 2022, Amarjit and coworkers formulated liposomes containing levonorgestrel using a solvent injection method. Particle size (147 ± 8 nm), polydispersity index (0.0207 ± 0.03), zeta potential (-23 ± 4.25 mV), drug loading ($18 \pm 3.22\%$), and entrapment efficiency ($85 \pm 4.34\%$) were recorded for

the developed liposomes. Researchers used cryo high-resolution transmission electron microscopy and cryo field emission gun scanning electron microscopy to observe the particles, finding that they had a smooth exterior and a spherical shape. Levonorgestrel demonstrated a stable pharmacokinetic profile and in vitro drug release throughout a 28-day span [132]. Li and his team in 2022 developed novel core-shell microneedle (MN) patch. The developed formulation showed controlled release of contraceptive hormone, levonorgestrel (LNG), for 6 months. An MN mold was utilized to sequentially cast a core of poly(lactide-*co*-glycolide) (PLGA), a shell of poly(L-lactide) (PLLA), and a cap of poly(D,L-lactide) (PLA) around LNG. The core-shell MNs utilized an effervescent interface to detach from the patch backing and begin to adhere to the skin in under a minute after application. The core-shell architecture enabled nearly zero-order LNG release over 6.2 ± 0.1 months in vitro while limiting the initial 24 h burst release of LNG to $5.8 \pm 0.5\%$. A monolithic MN patch containing an identical LNG and PLGA core but lacking the rate-controlling PLLA shell and PLA cap achieved LNG release for only 2.1 ± 0.2 months, with a larger LNG burst release of $22.6 \pm 2.0\%$ [133].

3.6 Treatment of diabetes

Insulin is a hormone produced by the pancreas' islet cells that aids in the regulation of blood sugar levels. Type 1 (which cannot make its own insulin) and type 2 (which cannot respond to normal levels of insulin) diabetics generally require long-term insulin injections. However, insulin's limitations make it hard to keep blood sugar under control [134]. Accidental under dosing can result in hypoglycemia, which can cause seizures, coma, and even death. Therefore, diabetics on insulin need to keep a close eye on their blood sugar levels at all times. The use of MNs to transport insulin is a promising and cutting-edge field of research [135]. Microneedles (MNs) can be utilized for wound healing owing to their ability to puncture the epidermis and deliver drugs directly to the wounded tissue beneath. Common MN patches, however, do not have adequate skin adhesion to keep the patch attached to the incision. Multilayer MN patches with adhesive back patching for tissue adhesion and diabetic wound healing were inspired by the barb hangnail microstructure of porcupine quills [136]. The polycaprolactone tips of MNs were loaded with sodium hyaluronate-modified CaO_2 nanoparticles and metformin (hypoglycemic drug), giving them potent antibacterial and hypoglycemic effects. To prevent the MN patches

from detaching from the application areas and transmitting bacteria, a flexible and adhesive back patching was produced by polyacrylamide-polydopamine/Cu^{2+} composite hydrogel. MN patches, with their bioinspired multilayer structure and desirable mechanical and antimicrobial qualities, represent a promising multifunctional dressing platform for accelerating wound healing [137]. Abd Azis and coworkers developed DMNs loaded with MTF in the form of glucose-responsive microparticles (GR-MPs) in 2022. Using a UV-visible spectrophotometer, an easy analytical approach was developed. Normal and hyperglycemic conditions were simulated by validating the procedure in four different media: phosphate-buffered saline (PBS), PBS containing 1% *w/v* glucose, PBS containing 2% w/v glucose, and PBS containing 4% w/v glucose. All of the media's calibration curves showed an R value (correlation coefficient) of 0.998, which is indicative of the method's linearity. Additionally, LLOQ values in the four different media ranged from 2.23 to 1.95 μg/mL, with a maximum value of 2.88 μg/mL [138]. In 2018, Migdadi and colleagues examined the feasibility of using a hydrogel-forming microneedle (MN) patch to provide prolonged transdermal delivery of the high-dose drug metformin HCl. This may reduce the risk of adverse effects in the gastrointestinal tract and the potential for absorption changes in the small intestine due to oral administration. The drug reservoir layer was lyophilized, and the MN layer was synthesized from an aqueous blend of 20% poly (methylvinylether-*co*-maleic acid) and 7.5% poly (ethylene glycol) cross-linked by esterification. Over >90% of the metformin was retrieved from the homogeneous drug reservoirs at 10,000 Da. The time it took for the drug reservoir to dissolve in the PBS (pH 7.4) was less than 10 min. Metformin HCl permeation across dermatomed newborn porcine skin was improved by MN in vitro. At 6 h, the 75 mg metformin HCl reservoir patch released 9.71 ± 2.22 mg, whereas the 50 mg patch released 10.04 ± 1.92 mg. After 24 h, the 75 and 50 mg metformin HCl reservoir patches released 28.15 ± 2.37 mg and 23.25 ± 3.58 mg, respectively. In contrast, a control setup containing simply the drug reservoirs resulted in a delivery of 0.34 0.39 mg after 6 h, 0.85 0.68 mg after 12 h, and 1.01 0.84 mg after 24 h. Following MN administration, metformin HCl was observed in rat plasma at concentrations of 0.62 ± 0.51 μg/mL 1 h after treatment and 3.76 ± 2.58 μg/mL 3 h later in vivo. After 24 h, the concentration peaked at 3.77 ± 2.09 μg/mL. The value of steady-state concentration (C_{ss}) was 3.2 μg/mL. It was calculated that 30% of metformin would be absorbed transdermally utilizing MNs [139].

4 Conclusion

Microneedles are a major transdermal drug delivery system, and their ability to impair the skin barrier function makes them very promising. These systems don't intact the skin's nerve endings, making its insertion painless. However, microneedles cause less anxiety than injections, which may boost compliance, especially in children. They release the medication at the stratum corneum-viable epidermis interface for local and systemic effects. Since vaccine dosages are low, microneedles offer considerable promising approach. Microneedles may be a simpler and more accessible method for mass immunization. Drug delivery research has traditionally used microneedles, and recent innovations have expanded their use and interest. These additionally presumed safety of microneedle-based techniques place such implants at the very forefront of biomedical as well as translational science. Despite encouraging results, industrial interest in microneedles is modest despite several research studies using a wide spectrum of drugs to treat diverse illnesses. Thus, research ought to strive to expand microneedles utility beyond immunization, offering more therapies and other intriguing options to the population. Microneedle technology could bridge the bench and clinic by satisfying patient needs and offering an alternative to conventional treatments.

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CHAPTER 2

Nanomedicine's delivery using microneedles

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In recent years, nanomedicine, which is defined as the application of nanotechnology and nanoscience for the prevention, diagnosis, and treatment of diseases, has progressed remarkably since its inception [1]. Research in the field of nanomedicine has led to the development of new materials that function as vehicles for the delivery of therapeutic or imaging agents with the ultimate goal of improving clinical outcomes. To achieve this objective, nanocarriers need to efficiently deliver therapeutic payload to diseased sites in the body with cellular specificity and even at the subcellular level, as well as allowing sustained drug release over a long period of time, thereby providing protection against degradation chemically or proteolytically to the encapsulated materials, and increasing their bioavailability. Therefore, nanotherapy overcomes the obstacles that limit conventional medicines and has the potential to meet future market demands for disease treatment [2].

The nanocarrier formulations can be administered by different routes, such as oral, intravenous, pulmonary, nasal, and ocular. The oral route continues to be the main route of choice despite the fact that it has several drawbacks, such as the first-pass effect, which can reduce plasma concentrations of the drug; and also, that some drugs lose stability in the gastrointestinal tract [3,4]. On the other hand, the parenteral route does not present all the limitations associated with the gastrointestinal route and allows a rapid onset of the therapeutic effect after the administration of a reduced dose of drug. However, this route presents disadvantages such as poor compliance by the patient due to the association of pain with the use of syringes and the need for trained personnel to administer the medication. An effective

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alternative to parenteral and oral delivery systems is the use of transdermal delivery systems, which are noninvasive systems that can be easily and painlessly administered to the patient. Additionally, nanotechnology has been explored in transdermal delivery to enhance the penetration of therapeutic agents through the skin. The skin is the organ that, besides protecting the human body, also works for the administration of drugs. The skin is made up of three main layers: the epidermis, composed mainly of the stratum corneum (SC); the dermis, which is the thickest layer; and finally, the hypodermis [5]. The SC acts as an important lipophilic barrier that reduces the number of drugs that can be delivered via this route. A novel strategy to overcome SC is the use of microneedle (MN), which has received enormous attention since the advent of microfabrication techniques [6]. The MNs are composed of arrays of micrometric-size needles. The dimensions of these microprojections generally range from lengths of only a few micrometers to lengths of up to 2000 μm, and they are grouped in a solid base or patch. They are minimally invasive and painful devices that can bypass the SC barrier without stimulating the dermal nerves due to the short length of the individual needles. In addition, macromolecules and small molecules can be administered. Holes created in the skin by MNs can be used to achieve drug delivery from the skin surface into the dermal microcirculation [7,8].

In recent years, extensive research has been carried out in the field of MN technology using different materials, MN designs, and manufacturing methods [7]. Moreover, the current progress on the development of the various combinatorial applications of MN in the area of nanomedicine will be discussed.

1 Stimuli-sensitive MN

Recently, stimulus-responsive MNs, mainly based on polymeric matrices, have been introduced to facilitate and control the release of therapeutic payload. Stimulus-responsive materials comprise a wide range of compounds that are capable of responding to changes in their surrounding environment (Fig. 1). The responses that the materials can present as a response to the stimulus can be dissociation of the formulation, degradation, swelling, and rupture of the matrix [9]. The stimuli to which this type of system can respond for the release of the therapeutic load can be internal, for example, pH, redox, enzymes, and glucose, and/or they can also be external stimuli, for instance, temperature, electric field, light, and mechanical stress. Spatiotemporal control of response systems improves the therapeutic efficacy and reduces the potential side effects associated with off-target drug delivery [10].

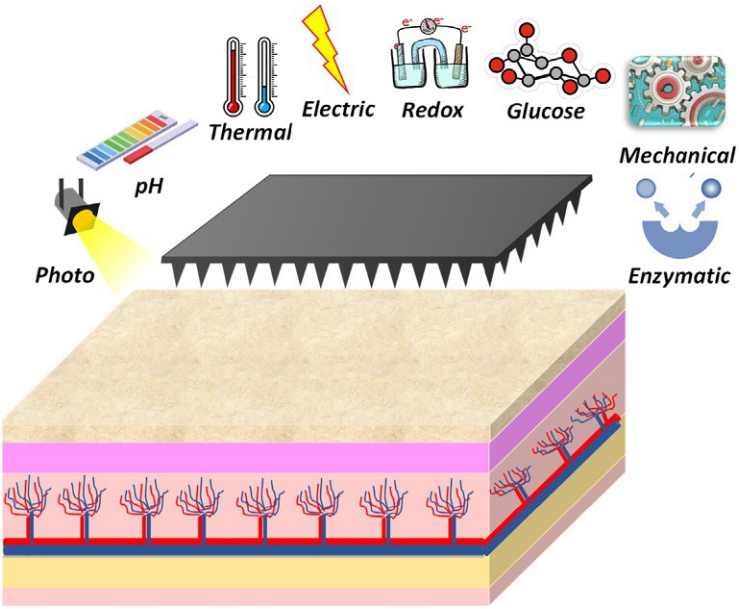

Fig. 1 Different types of internal and external stimuli for delivery of drugs in transdermal MNs.

2 MN activated by external stimuli

The construction of responsive MNs requires the incorporation of domains that can be activated by external stimuli. The development of light-sensitive NMs made by micromolding has been reported; these were built with a polymer (pHEMA) prepared from 2-hydroxyethyl methacrylate (HEMA) and ethylene glycol dimethacrylate (EGDMA), which can form a hydrogel. A light-sensitive conjugate between ibuprofen (IBU) and 3,5-dimethoxybenzoin was incorporated into the polymer matrix (Fig. 2). The degree of swelling of the polymer was 50% after 24 h compared with other hydrogels, which exhibit a lower and slower swelling, which are useful conditions to produce prolonged drug delivery systems. The mechanical tests carried out on the MNs showed minimal deformation when the insertion of these into the skin was simulated. When the array with light-sensitive conjugate-loaded MNs is irradiated with 365 nm wavelength light, the ester bond of the IBU conjugate is cleaved, producing free drug. The highest concentration of IBU was carried out in three cycles of irradiation with light, and no release of IBU was observed in the intervals where the system was "off," that means without irradiation. Therefore, this technology has a potential use in situations in which the administration of drugs "on demand" is required [11].

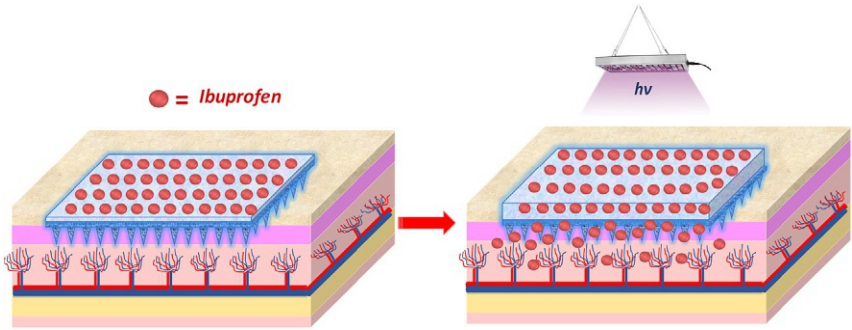

Fig. 2 Graphical presentation of ibuprofen delivery by MN using light stimuli.

In another work, the design of near-infrared (NIR)-sensitive MNs built with a poly(vinylpyrrolidone) (PVP) polymer was reported, which contained hollow mesoporous silica (SiO_2) nanoparticles (HMSN) loaded with the hypoglycemic drug metformin (MET) and coated with polydopamine (PDA)/lauric acid (LA); the former will respond to NIR stimulation, while the latter will change its phase with photothermal conversion of PDA under NIR light; this will allow the release of MET. It was found that under NIR irradiation, the LA coating exhibited clear thermal ablation due to photothermal transfer of PDA and thus, promoted the release of HNSN-encapsulated MET. In addition, this drug delivery system could release charged MET in "on-off" cycling delivery in the presence of the NIR laser, and it will decrease the risk of hypoglycemia [12]. In another work with MET, another external stimulus, temperature, was used. Zhang et al. [13] fabricated separable MN by molding. The solid support matrix was constructed with polyvinyl alcohol (PVA), and arrowheads were prepared with polycaprolactone (PCL), which were thermally ablated and contained MET. When MNs embed into the skin, the solid support matrix dissolves, and the arrowheads are separated under the skin. When the arrowheads are exposed to an electrical heating sheet, PCL MN will melt, turning from a solid to a liquid state, increasing the mobility of the polymer chains and allowing the release of MET. This MN compound system allows to thermally modulate the release of encapsulated MET, permitting on-demand control and controlling the dose of released MET, thereby improving treatment efficiency and reducing side effects.

3 MN sensitive to internal stimuli

Internally responsive MNs incorporate polymers that are sensitive to the occurrence or variation of a biological signal (e.g., pH, reactive oxygen species (ROS), glucose, or enzymes). Depending on the involved pathology,

researchers can choose different internal triggers [10]. In a work reported by Yu et al. [14], a removable transdermal patch of MNs constructed of a glucose-sensitive polymer matrix, loaded with insulin, was developed. The MNs were constructed using in situ photopolymerization. Under hyperglycemic conditions, the phenylboronic acid (PBA) units contained in the polymeric matrix reversibly form complexes with glucose, which are negatively charged, causing swelling of the polymeric matrix, which weaken the electrostatic interactions between insulin and polymers, thus promoting the release of insulin. In the design of another more complex glucose-sensitive MN system, MNs integrated with mesoporous silica nanoparticles (MSNs) are presented, which were modified with pinacol ester molecules of 4-(imidozoylcarbamate)phenylboronic acid (ICBE) and formed an inclusion complex (CI) with α-cyclodextrin (αCD). Glucose oxidase (GOx) and insulin were encapsulated in the MSN, and insulin retention within the MSN was achieved by IC formation. The modified MSNs were dispersed in the polymer matrix, and the MNs were constructed by molding. Once MN inserts into the skin, GOx will convert glucose to gluconic acid and hydrogen peroxide (H_2O_2) will be produced, which will oxidize ICBE and be released from the IC to liberate the preloaded insulin. In vivo tests demonstrated that MN released insulin is mediated by glucose levels and sensitive to H_2O_2 [15].

On the other hand, an MN patch was designed for the treatment of obesity in patients with type 2 diabetes. The MNs were constructed of dextran degradable cross-linked matrix, which contained dispersed nanoparticles (NPs) loaded with the Rosi drug. In addition, they contained GOx and catalase to produce an acidic environment due to the degradation of glucose by GOx and to promote matrix degradation. In vivo studies demonstrated a systemic increase in energy expenditure and fatty acid oxidation, effective control of body weight in diet-induced obese mice, as well as improved insulin sensitivity [16].

One promising strategy that has been studied is the design of pH-sensitive MN that can function as safe and painless vehicles for transdermal delivery of vaccines for potential cancer therapy. MNs were built with polycarbonate coated layer by layer with a pH-sensitive copolymer, which when found at acidic pH is negatively charged, and poly(I:C) that will function as an immunostimulatory adjuvant. This coating of the MN allows the effective loading of nanoengineered DNA polyplexes. When the MN device is implanted into the skin, the coating copolymer will be influenced by the pH and will transform into anionic copolymers, thus releasing the DNA vaccine, due to electrostatic repulsion. When this MN system was

tested in vivo, it was observed that it could induce a humoral and cellular response capable of inhibiting the growth of murine melanoma cells; therefore, it can be beneficial for cancer therapy [17].

In another study, PVP MNs containing pH-sensitive hollow microspheres of (D,L-lactic-*co*-glycolic) acid copolymers (PLGA HMs) were developed. Two drugs were used for simultaneous administration of this drugs. PVP MNs were loaded with the first drug, a green fluorescent agent, Alexa 488; whereas, the PLGA HMs contained in their aqueous core the second drug, a Cy5 cyan fluorescent agent, and sodium bicarbonate ($NaHCO_3$). After the system was inserted into the skin, the PVP MNs dissolved significantly within minutes releasing the first drug along with the PLGA HMs. Once in the acidic environment of the skin, protons (H^+) could freely pass through the PLGA shell membrane and react with $NaHCO_3$ and generate a large number of CO_2 bubbles, which would create holes that would rupture the membrane of the spherical shell of PLGA, thus releasing the second drug encapsulated in the microspheres. This approach can be clinically used for sequential and transcutaneous coadministration of a wide range of drugs [18].

In addition to the internal stimuli described previously for the design of MN, thrombin has been used to design a stimulus-sensitive MN system that functions as an anticoagulant. MNs were constructed with hyaluronic acid (HA) to which heparin-conjugated peptides were attached. When thrombin is present, the peptide can be cleaved, triggering the release of heparin, which is capable of inhibiting coagulation activation by inactivating thrombin. The activation/deactivation of this system is regulated by the presence of thrombin. When there is no thrombin, heparin will not be released, and therefore, the risk of undesirable spontaneous bleeding associated with conventional treatments is minimized [19].

Finally, Mir et al. designed a system of MNs composed of the polymer poly(ε-caprolactone) (PCL) sensitive to bacterial lipase for the treatment of infected chronic wounds. Inside the MN, there were PCL NPs loaded with carvacrol (CAR), an antibacterial agent. MN are capable of delivering NP directly to the infection site where they will be degraded by bacterial lipases and selectively deliver CAR to diseased tissue. In addition, NP can be retained on the skin for longer, a duration that could result in a sustained desired antimicrobial effect by avoiding multiple applications [20].

4 Gene therapy with MN

Gene therapy is a promising technology for the treatment of diseases by transporting and delivering genetic material to a specific cell with the aim of correcting or compensating genetic defects [21]. DNA and RNA are short oligonucleotides, basically smaller units than proteins. In recent years, DNA and mRNA vaccines have been shown to present a rapid response to emerging infectious diseases as safe and useful treatment options. In addition, nucleic acid therapies have been shown to have great potential for the treatment of other types of diseases, such as cancer and some skin diseases, which other treatment approaches have not been able to achieve [8,22].

Despite its potential, the delivery of nucleic acids to target sites in the cell, such as the nucleus and cytoplasm, faces several obstacles because it is a charged macromolecule. For example, if transdermal delivery of genetic material is required, it must cross tight junctions in the skin and then resist degradation by endogenous enzymes until it is internalized into the cell or nucleus. The application of MN to deliver genetic material therapies through the skin has been explored with different delivery approaches [22,23]. The design and preparation of PVP polymeric MN for the delivery of NPs composed of a peptide capable of transporting DNA-encoding human papillomavirus (HPV) antigens as a potential vaccine were reported. Results of immunogenicity tests revealed that the vaccine was able to have a better antigen-specific response than intramuscular (IM) administration. Moreover, the NP-loaded MN vaccine protected against the development of cervical tumors and delayed tumor progression. These vaccines also have the advantage that they do not require cold chain storage, which significantly reduces the costs of the pharmaceutical product [24].

Continuing with the theme of delivery of genetic material with MN, the preparation of a multilayer MN system of a polyelectrolyte of heparin and albumin (PMA), which can support DNA polyplexes directed to antigen-presenting cells (APCs) as a potential vaccine for Alzheimer's disease, was performed. The MN system demonstrated rapid release of DNA polyplexes after application to the skin. Release of the polyplexes increased their potential to contact and transfect intradermal APC. This DNA vaccine encodes a secretable Aβ fusion protein as an antigen for the treatment of Alzheimer's

disease. The results show that this MN vaccine is a better option in terms of administration, transportation, and storage, than a vaccine administered subcutaneously [25].

5 MN for ocular drug delivery

The eye is the organ responsible of the perception of the visual world, associated with the reaction to light, the distinction of colors, and the visual estimation of shape and distance. Diseases that occur in the eye can be divided into two main groups: diseases of the anterior segment of the eyeball, which include deficiencies of the cornea, conjunctiva, ciliary body, and lens; and posterior segment diseases involving the sclera, vitreous humor, retina and choroid, and optic disc (optic nerve). Posterior segment diseases can lead to permanent vision loss, if left untreated, and account for most of the blindness. Drug administration to the eye can be performed by multiple approaches, such as systemic, topical, periocular, and intravitreal routes, which must face certain barriers that cause short ocular retention time, reduced drug accumulation, insufficient bioavailability, and can become highly invasive pathways.

To achieve targeted drug delivery to the eye, the use of MN has recently been proposed as a minimally invasive and effective alternative. Additionally, MN can be used for the treatment of anterior and posterior segment diseases. Being minimally invasive, the technique reduces pain, tissue damage, and the possibility of bacterial contamination associated with hypodermic injections [26,27]. The administration of MN combined with nanocarriers represents a promising model to selectively deliver and concentrate drugs in ocular lesions [28].

In the design of MN for ocular administration, polymeric MN and hollow MN are commonly used. Jiang et al. [29] developed an MN system to deliver drugs through the sclera. The MNs were hollow and loaded with polylactic acid (PLA) nanospheres or latex microspheres for sulforhodamine infusion. The results showed that the nanospheres could be delivered through the MN by insertion-retraction, while microsphere delivery required the addition of an enzyme to alter the sclerotic tissue structure. The study demonstrated that hollow MN function to deliver nanosystems into the sclera in a minimally invasive manner. In another work, the design of MN to deliver NPs and microparticles (MP) into the suprachoroidal space was reported, which can be used as a minimally invasive and targeted method to deliver drugs in a sustained manner to the posterior part of the

eye, specifically toward the choroid and the retina. The study demonstrated that MNs were able to deliver sulforhodamine B solution and suspensions of MP and NP into the suprachoroidal space of rabbit, pig, and human eyes. Administration into the suprachoroidal space represents a better solution over current periocular and intravitreal delivery methods, which are not well targeted for posterior segment diseases. In addition, in this study, they demonstrated that MN length, particle size, and infusion pressure affect the success rate of particle delivery to the administration site. The observed results indicate that the smaller particles (20–100 nm) flowed more easily through the scleral tissue because they could more easily pass through the collagen fibers. Also, the success rate increased as the length of the MN augmented, since the amount of scleral tissue between the MN and the suprachoroidal space was reduced [30].

In other work, an NP-loaded bilayer solution MN matrix system was developed to sustainably deliver macromolecules, such as proteins. The NPs were made of the PLGA polymer and encapsulated ovalbumin (OVA) and were prepared by a double-emulsion method. OVA was selected as a model protein to study whether MNs were capable of releasing molecules of this size. Polymeric NPs were embedded in a PVA polymer matrix to construct bilayer MN. The results showed that the MNs were mechanically stable and could perforate the sclera, then degraded very quickly, releasing the NP in less than 3 min. NPs were able to release OVA in a sustained manner for more than 2 months. The ex vivo studies demonstrated that the design of these NPs resulted in a greater depth of distribution and retention time of the NPs in the scleral tissue, so that they could be used to deliver drugs in the posterior part of the eye [31].

6 MN for cancer treatment

Despite all the efforts made to develop new cancer treatment strategies, it continues to be one of the main health problems affecting the world population; chemotherapy and immunotherapy remain important options for the treatment of this disease. Several drug carriers have been investigated to improve the therapeutic efficacy and bioavailability of anticancer agents. However, systemic administration of anticancer treatments can trigger serious side effects [8].

MN technology generated a new alternative in cancer therapy through the efficient administration of antitumor therapies. Various chemotherapeutic agents, genes, and proteins can be efficiently delivered via MN-based

devices. In this way, the local application of MN-based systems can increase the distribution and amount of drug reaching the deeper regions of tumors, while minimizing side effects. Moreover, MNs also allow the combination of different drugs in a single therapy and their time-controlled release, which can be used to enhance the antitumor effect [32].

In a work reported by Liao et al. [33], a nanosystem, M@DGP, was designed based on MSN containing the anticancer drug, doxorubicin (DOX), and gallic acid-iron (GA-Fe) complexes, which can induce the generation of ROS. In addition, MSNs were covered with a layer of polydopamine, which provides a response to photothermal therapy (PTT) and the capacity to consume glutathione (GSH). M@DGPs were included in an easily dissolved MN system for the treatment of melanoma; MN will increase the accumulation of M@DGP at the tumor site. The system demonstrated that once MNs were inserted into the skin, they quickly dissolved and promoted the release of M@DGPs, which could cause ROS accumulation within tumor lesions and GSH depletion, achieving efficacy superior antitumor [33]. Another work combined chemotherapy and PTT to ensure simultaneous delivery to the tumor site of the chemotherapeutic drug and the photothermal agent. MN patches containing DOX-loaded MSN and indocyanine green (ICG) were prepared. The results showed that the MN patch exhibited good skin penetrating ability, and the tips of the patches dissolved to release the DOX- and ICG-loaded MSNs at the tumor site. When an 808 nm laser was applied for 2 min, the local temperature in the tumor quickly reached 48°C. Therefore, the combination of chemotherapy and PTT in MN/MSN DOX-ICG patches induced a better antitumor effect in human osteosarcoma (MG-63) cells [34].

On the other hand, the MN design for the transdermal delivery of an antitumor vaccine was reported. The MNs were constructed with an amphiphilic triblock copolymer, which, after cutaneous application of the MN, dissolve and form nanomicelles in situ. The nanomicelles facilitate the encapsulation of the hydrophobic toll-like 7/8 receptor agonist R848 and the hydrophilic OVA antigen. Nanomicelles demonstrated efficient delivery of R848 and OVA to lymph nodes and promotion of uptake by APC, resulting in significant antitumor activity [35].

Improving the administration of chemotherapeutic drugs to reduce the side effects associated with their low specificity is crucial [36]. In a work reported by Lan et al. [37], they were constructed by MN molding to mediate transdermal delivery of lipid NPs loaded with the chemotherapeutic agent, cisplatin (CP). Local delivery of CP using pH-sensitive lipids

combined with the MN solution increased the solubility and delivery efficiency of CP. In vivo studies in a xenograft animal model of head and neck cancer demonstrated that local delivery allowed for increased cellular uptake and thus, improved cytotoxicity and increased apoptosis in cancer cells, resulting in a significant reduction in the volume and weight of the tumor. In addition, no damage to nontarget organs was detected, so that the designed system managed to increase the efficacy and safety of CP.

As mentioned previously, DOX is widely used as an effective chemotherapeutic drug to treat various types of cancer. However, the drawbacks associated with its administration that DOX presents limit its clinical application. Therefore, DOX has been encapsulated in various nanomaterials to decrease nonspecific toxicity and increase anticancer efficacy. Recently, these nanosystems have also been used to manufacture MN for the treatment of cancer [36]. Yang et al. developed a HA-based dissolution MN system, which contained transferosomes for the release of DOX (DOX-T). The complexes (DOX-T/MN) were administered through the lymphatic route. Study of this system demonstrated that MNs were able to successfully insert into rat skin and release DOX-T into the dermis through self-dissolution. In addition, DOX accumulation in lymph nodes was promoted compared with a conventional transdermal administration [38]. In another work to further study DOX delivery with nanosystems and MN, the use of MN Derma roller in combination with DOX-loaded liposomes and celecoxib (CEL) was investigated as a potential therapeutic approach for melanoma. DOX penetration into the skin was increased by approximately two times with this system compared with passive delivery. The results demonstrated that the liposomes significantly enhanced the antitumor effect. In addition, treatment with Derma rollers before application of the liposome gel improved the rate of tumor inhibition [39].

In an alternative used as chemoprevention for breast cancer, CEL is used as a treatment to inhibit the enzyme cyclooxygenase 2 (COX-2), which has been found to be present in situ in breast biopsies of atypical hyperplasia and recurrent ductal carcinoma. Although clinical trials have suggested the efficacy of orally administered nonsteroidal antiinflammatory drugs as chemopreventive agents, cardiovascular risks have made their use difficult. Topical application to the skin of the breast can overcome these problems. For this reason, microemulsions were designed as topical delivery systems for CEL. To enhance the delivery of the microemulsion, MNs were used. The results supported that the combination of the microemulsion with the MN was essential to improve the penetration of CEL [40,41].

7 Combining NP with MN for vaccine delivery

For many years, the prevention of contracting a disease involves the inoculation of a biological agent (vaccine) in the organism to stimulate the immune system of the body and provide protection before a first or future infectious biological agent encounter. Therefore, a vaccine is a biological preparation that provides active acquired immunity to a particular disease. In this sense, vaccine is designed with a killed or weakened form of disease-causing biological agent, its toxins, or one of its surface proteins [5].

In general, vaccines are frequently administered using hypodermic needles, needing personal expert for the administration, and regarding vaccine, a cold chain storage during the transportation of liquid formulations is strictly necessary. On the other hand, some vaccines are lyophilized, diluted, and injected multiple times, which inevitably increases the cost and waste, as well as the probability of contamination during transportation and storage [42]. Oral delivery largely overcomes these problems, but many drugs and, of course, one vaccine cannot be given by this route due to poor absorption and degradation in the gastrointestinal tract and liver [43]. Other routes of administration have also been investigated, but none of them offer the same effectiveness of direct injection using a needle [44].

As a micron-scale device, an MN should be large enough to deliver almost any drug or small particulate formulation, but still be small enough to avoid pain, fear, and the need for expert training for administration. Moreover, an MN allows precise tissue localization of delivery, such as within the skin, the suprachoroidal space of the eye, and the cell nucleus [44]. Furthermore, the use of MN also ensures a stronger immune response due to sustained administration [45].

Most applications of MN studied to date have emphasized drug and vaccine delivery to the skin. Conventional transdermal delivery is limited by the barrier properties of the outermost skin layer, the SC [46]. Various chemical, biochemical, and physical methods have been studied to increase skin permeability. However, chemical and biochemical methods do not appear to be broadly useful for delivery of biotherapeutics and vaccines across skin [44]. In this way, MN can be prepared as a low-cost patch that is simple for patients to apply for delivery of biomacromolecules. Targeting vaccine delivery to APCs in the skin using MNs is also of particular interest [44,47].

The issues of poor vaccine transport through the skin barrier, patient compliance, and strict storage conditions can be addressed by MNs. MNs can painlessly bore the SC and canalize the epidermis to improve the

vaccination [48]. Additionally, MNs should be regarded as a unique strategy for the delivery of antigen to immune cells such as dendritic cells within the skin, which is an essential problem in traditional vaccine delivery. So far, MN reports have demonstrated similar or even higher immunogenicity and dose sparing [49]. Several investigations have reported the application of different MN strategies and their use in various vaccine formulations, including influenza and HPV [8]. Administration of anthrax and rabies vaccine using hollow MN was also studied [50]. Ogai et al. [51] fabricated hollow MN from polyglycolic acid to enhance the vaccination efficiency by the intradermal route. The precise delivery of the biological agent in the upper dermis provides enhanced immunity [5]. After the vaccination, the antibody titers were significantly higher with intradermal vaccination with MN as compared with subcutaneous injection on 15th day [51]. Dissolving MNs were also investigated for intradermal vaccination [52].

A dissolvable MN is a common type of MN used for vaccine delivery purposes [53]. The dissolvable MNs can be used to replace hypodermic injection needles (Fig. 3). Unlike other types of MN, the dissolvable MNs are biocompatible, robust, scalable, and do not generate biohazardous waste [54]. Dissolvable MNs were used to deliver vaccines for malaria, diphtheria [55], influenza [56], Hepatitis B [57], HIV [58], and polio [59].

Even though dissolvable MNs are most frequently used for vaccine delivery, coated MN arrays have also been successfully used for vaccination purposes [53]. A study used a simple, safe, and compliant vaccination method to improve the immune system for pigs by administering bacillus Calmette-Guérin vaccine with a coated MN [60]. Hollow MNs have been used to

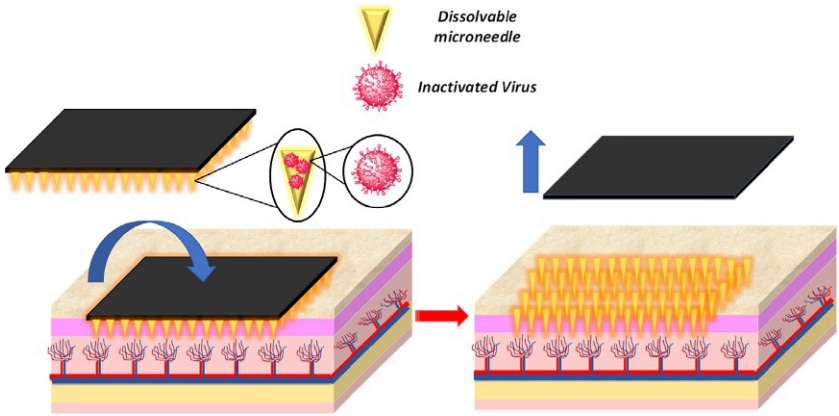

Fig. 3 Design of a dissolvable MN for vaccine delivery.

deliver anthrax recombinant protective antigen vaccine to a rabbit instead of regular injection [61]. A hollow MN was evaluated for vaccination against plaque in a mouse model [62]. A clinical trial conducted in humans using hollow MN with influenza vaccination showed similar results with the immune system when compared with intramuscular injection [63].

Recently, the incorporation of vaccines into particle-based systems has been proposed as a novel immunization strategy for the successful delivery of vaccine therapeutics [64]. The use of particle-based vaccination systems can aid in the stabilization of vaccine antigens in vivo, in addition to providing controlled and sustained release of the same at the administration site. NPs have been shown, by a variety of different research groups, to display inherent immunogenic properties. For elaboration, it has been shown that induction of T-cell immune responses against encapsulated antigens can be achieved using NP technology, with the NPs used in the studies themselves exhibiting immunogenic properties, comparable with those of traditional adjuvants such as aluminum hydroxide and Freund's complete adjuvant [65,66]. Using NP technology, antigenic material can be adsorbed or conjugated onto the surface of the particles or directly incorporated within the polymeric matrix [67]. Further advantages of the use of antigen/NP formulations include the fact that such formulations protect labile antigens from proteolysis, prolong the uptake of antigenic material by antigen-presenting cells, and reduce the release of antigen into systemic circulation [68].

Among the first examples appearing in the literature that combine the use of NP formulations in conjunction with MN devices, there is the work of Bal et al. [69] that demonstrated the effective delivery and immunogenicity of *N*-trimethyl chitosan (TMC) adjuvanted diphtheria toxoid (DT). These DT-loaded TMC NPs were coated onto solid metallic MN arrays, ultimately facilitating their delivery to the skin layers. TMC has a permanent positive charge and is, therefore, soluble in water over a wide pH range, which allowed the researchers to efficiently create DT-loaded NPs.

On the other hand, ovalbumin (OVA) is a widely utilized model antigen in vaccine delivery studies. In this regard, liquid formulations of OVA or OVA-conjugated NPs were applied to the skin surface jointly with roller MN devices, serving as a means of enhancing transdermal delivery [11]. Again, TMC NPs were used in this mouse study. As a result, significantly higher anti-OVA IgG titers were recorded in those mice, which had been treated with OVA-conjugated NPs, in comparison to those that had been treated with free OVA solution [70].

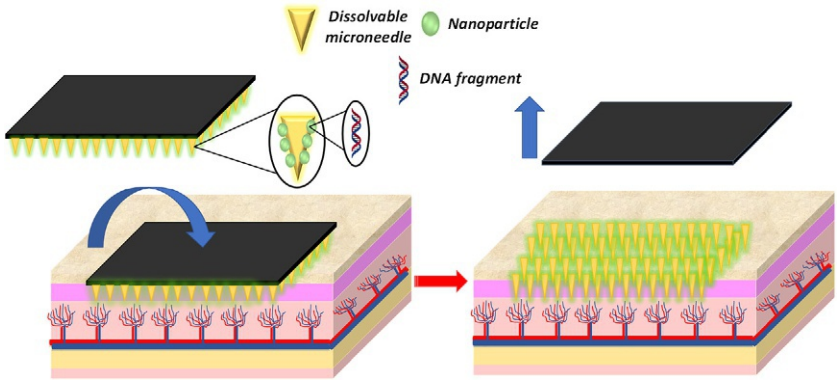

Fig. 4 Design of NP charged with DNA in MN for improvement of the conventional vaccines.

Although protein-based antigens have been the mainstays of vaccination methodologies for many years, novel DNA vaccines have and continue to gain significant attention in the scientific literature. To this end, expanding the remit of NP formulations to incorporate DNA vaccines for transdermal delivery is a logical next step [51] (Fig. 4). In this regard, Kumar et al. [71] reported on plasmid DNA-coated cationic PLGA NPs. Through a solid MN roller device that was used in conjunction with the NP formulation, but, in this case, the NPs were coated with plasmid DNA. This unique means of DNA vaccine delivery resulted in the successful immunization of mice with a gene encoding a protective antigen against anthrax. An interesting outcome of this study was the fact that it highlighted how positively charged plasmid DNA-coated NPs elicited a stronger immune response in mice than negatively charged plasmid DNA-coated NPs or plasmid DNA alone [51].

As we have been mentioning, the main reason for the use of NPs in combination with MNs to deliver the antigen is the improved stability and controlled release of antigen for inducing higher immunogenicity. Another example reported is the encapsulation of chicken OVA into PLGA-NPs, which were then incorporated in dissolvable MNs [50]. Through this approach, MNs slowly released the antigen to lymph nodes occupied with dendritic cells. This strategy led to the successful in vivo immune system activation against influenza and melanoma tumors [72]. In another study, monophosphoryl lipid A, OVA, imiquimod, and toll-like receptor agonists were encapsulated in PLGA NPs, which were then intradermally delivered through hollow MNs. Unlike classical intramuscular injection, the MNs generated higher levels of IgG2a antibody and IFN-γ-producing

lymphocyte [73]. In this regard, Guangsheng et al. [74] compared different types of nanocarriers to modulate the immune response by hollow MNs. To do so, OVA was loaded into mesoporous silica NPs, liposomes, PLGA, or gelatin NPs with or without polyinosinic:polycytidylic acid as an immunostimulant. Liposome and PLGA induced significantly higher IgG2a response. Moreover, liposomes led to $CD4^+$ and $CD8^+$ T-cell activation.

Finally, it is important to mention that there is solid evidence on the advantages in the use of MN devices for the delivery of biological agents to the skin. Therefore, combining this technology with the many positive aspects of NP formulation, specifically in terms of the potential for prolonged antigen stability, depot release, and the augmentation of immune responses, serves to make this combinatorial strategy an enticing and exciting area for future research [51].

8 Improved delivery of analgesics using NP-modified MNs

The International Association for the Study of Pain (IASP) describes pain as an aggressive sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage [75]. This definition suggests that pain can also be perceived in the absence of tissue injuries. It also identifies pain to be instinctive as perception of pain differs in everyone depending on their emotional, sensory capacity, and experiences with previous injuries. Pain can be classified in many ways; however, the most common type of pain is the nociceptive pain that arises when a noxious stimulus is detected that leads to the activation of the nociceptors [76,77]. These are the sensory receptors of the peripheral somatosensory nervous system expressed throughout the body, including the visceral tissues that respond to mechanical, thermal, and chemical stimulus. Activated nociceptors initiate a neural process of encoding the stimulus leading to a signal transduction from the peripheral to the central nervous system [78]. Depending on the site of origin, nociceptive pain can be categorized into three types, such as cutaneous, somatic, and visceral [79]. While cutaneous and somatic nociceptive pain is constant and typically well localized in a small region, visceral pain originates from internal organs, is episodic and not confined to one region.

Nociceptive pain is characteristically acute and temporary; however, depending upon the conditions, it can become chronic [80]. The current research focuses on developing a new drug delivery approach for the management of cutaneous nociceptive pain. For these reasons, the treatment

approaches for cutaneous nociceptive pain require understanding of the stimulus that leads to nociceptor activation [79]. Cutaneous drug administration in the form of creams, spray lotions, or dermal patches directly at the peripheral site of origin of the pain can deliver relevant drug concentrations at lower drug loading with minimal plasma exposure and without compromising the therapeutic effectiveness. This approach reduces adverse effects that are encountered with various conventional formulations [81].

However, the barrier properties of the skin limit absorption of drugs after local application. Drug permeation across the skin is a slow process and often requires multiple application regimens to attain therapeutically effective concentrations required for pain relief [81]. Additionally, when immediate pain relief is desired, conventional formulations cannot deliver the drug fast enough to provide a quick onset of action. In the event when rapid drug concentrations are required locally into the skin, breaching the skin barrier is an effective technique [78,82].

The transdermal route is eminent for its painless distribution. Among transdermal drug delivery systems, MNs are gaining attention for their application with delivery at the deeper dermal layer because it bypasses the major barrier of the skin, easily accesses the skin dermal microcirculation, prevents damage to dermal blood vessels, and can be simply inserted into the skin without using any additional applicator devices [83].

The analgesic medications for the fight against pain can be classified into three general categories according to the action site of the pain pathway [84–86]: (1) opioid analgesics usually acting on the central nervous system for relieving severe pain [87], such as morphine, codeine, meperidine, and fentanyl [88]; (2) nonopioid analgesics such as nonsteroidal antiinflammatory drugs (NSAIDs) mainly prevent prostaglandin synthesis by inhibiting the enzymes cyclooxygenases 1 and 2 (COX-1 and COX-2), therefore exerting peripheral effects for treating pain [89]; (3) adjuvant analgesics mostly changing the way nerves process to manage pain [90] are illustrated as "meditation whose primary indication is the treatment of a medical condition, with secondary effects of analgesia." Though opioid and nonopioid analgesics are the main drugs used to treat pain, adjuvant analgesics are widely applied as an auxiliary in first-line pain treatment for chronic or neuropathic pain [91].

The oral administration of analgesic medications such as NSAIDs has the tendency to incur adverse effects in the digestive tract caused by their inhibition capacity over the constitutive COX-1. Intramuscular and intravenous injections of opioid analgesics and adjuvant analgesics are concomitant with

pain and require medical expertise for administration. MNs as the supplement of oral administration and injection have been explored for delivering analgesic medications for pain management [92].

Opioid MNs holding sustained effects have the potential to avoid peaks and troughs of intermittent dosage regimens of injections. Abdalla et al. [92] investigated tramadol-coated MNs treating temporomandibular joint inflammatory pain and evaluated the difference of therapeutic benefit between tramadol injection and tramadol-coated MNs. The results turned out that the two ways of administration had the same antinociceptive effect and antiinflammatory effect, but the tramadol-coated MNs had a more durable antinociceptive effect (2 days) than injection (2 h). When tramadol solutions are injected, the solutions can leave the injection site quickly due to convective flow. In contrast, the coated MNs deliver tramadol in solid phase, and it takes relatively long time to diffuse away, which creates a high local concentration of tramadol for a long duration [92].

Opioid MNs can relieve pain rapidly by accelerating the drug onset time. Compared with patches, opioid MNs possessing the same analgesic effect but with fewer drug loadings have the possibility to avoid drug residues. Maurya et al. [78] evaluated the regional antinociceptive activity of fentanyl dissolving MNs. The dissolving MNs were composed of HA and fentanyl. The animal antinociceptive activities of fentanyl MNs and fentanyl patch were estimated by hot-plate analgesia method. It turned out that fentanyl MNs had a faster onset time (0.5 h) than the patch (6 h) and fentanyl MNs with relatively fewer drug loadings compared with the patch providing an effective antinociceptive activity by measuring the paw withdrawal latency of rats. The results indicated that fentanyl MNs could manage immediate pain effectively.

On the other hand, dexamethasone is an important steroidal medicament used to treat the inflammatory disorders of the eye such as diabetic macular edema, retinal vein occlusion, and noninfectious posterior uveitis [93–95]. Drastic increase in intraocular pressure and other side effects such as cataract and risk of infections are observed as posttreatment symptoms when dexamethasone is administered in the form of injections, meaning that the acute side effects were predominantly due to the mode of administration or the formulation content and not due to the drug [96–98]. In this regard, Matadh et al. [98] proposed the use of the polymer-coated polymeric (PCP) MNs as a novel approach for controlled delivery of drugs without allowing release of the excipients to the target site. Therefore, PCP-MNs were explored as an approach to deliver the drug intravitreally to minimize the risks associated

with conventional intravitreal injections. In this design, the core MNs were fabricated with polyvinyl pyrrolidone K30 (PVP K30) and coating was with Eudragit E100. Preformulation studies revealed that the films prepared using Eudragit E 100 exhibited excellent integrity in the physiological medium after prolonged exposure. As a result, the drug release from uncoated MNs was instantaneous and complete. On the other hand, a controlled release profile was observed in case of PCP MNs. Similarly, even in the ex vivo porcine eye model, the drug release was gradual into the vitreous humor in case of PCP MNs. The uncoated MNs released all the drug instantaneously where the PCP MNs retarded the release up to 3 h.

Rheumatoid arthritis (RA) is a common chronic autoimmune disease and eventually ends in severe disability and death. RA has no cure, but there are some treatments for decreasing the severity of various symptoms: for example, pain management of RA by NSAIDs, neutralization of the inflammatory cytokines (or block their functions) of RA by specific biologics [99]. However, the clinical benefit is limited due to their low permeability and poor bioavailability. Meanwhile, the system toxicity for long-term use of NSAIDs is another focal issue. To address this, Du et al. [100] fabricated melittin-loaded HA MNs and melittin-loaded methacrylate-modified HA-MNs. The in vitro release study showed that the melittin-loaded HA-MNs released complete melittin within 10 min, and melittin-loaded methacrylate-modified HA-MNs showed burst release of 56% within 10 min, and the rest were released till 480 min. MN administration reduces the levels of IL-17 and TNF-α and raised the proportion of regulatory CD4 T cells, according to cytokine cell analyses in the blood serum and paws. The results from mouse and rodent models, in which RA was gradually introduced, demonstrated a decrease in paw edema and arthritis. This study demonstrated that the polymeric MNs are a potential transdermal delivery technique for melittin whose continuous release has the potential to improve therapeutic effectiveness even further while lowering the frequency of administration [83].

In the most recent works, Lin et al. [99] reported that a degradable biopolymer MN patch was designed and synthesized. In this dual drug delivery patch, NSAIDs were encapsulated by polymeric NPs, which were coated by the neutrophil membrane and then loaded into the MN patch for local transdermal delivery. When applied to the murine models, the MN patch gradually dissolves and releases the contained NPs. Coating of neutrophil membrane provides the nanosized NSAIDs with the capability of cytokine binding and inflammatory joint tropism. Based on inflammatory

microenvironment regulation by cytokine absorption and COX-2 inhibition, the MN shows a significant local antiinflammatory therapeutic effect on mouse model of collagen-induced arthritis and rat model of adjuvant-induced arthritis. Further development based on this advanced strategy may provide additional therapeutic options for RA treatment.

9 Delivery of cosmetics using NP-modified MNs

Wide variation in the materials used to fabricate the MNs for different applications leads to the fabrication of various MN designs [101]. As aforementioned, MNs have many advantages over other forms such as needle injection. Faster healing is observed at the injection site compared with the hypodermic needles; hence, postadministration skin is less prone to microbial infections in these cases. It does not require an expert for administration. It punctures the epidermis and is modified to penetrate up to any layer of the skin, with a reduced dose, good tolerability with long-term administration, and delivery of the payloads with a controlled rate as compared with the approaches meant for drug delivery through the SC [102,103].

In recent years, cosmetic combined with MN treatment has made a great progress. The fact that many cosmeceutical products were manufactured proves that cosmetic application is promising. In general, cosmetic applications are mainly divided into two parts. One is to promote the natural healing of the injured skin. The other is to enhance skin permeation of cosmeceuticals [56]. Minimally invasive delivery of MN created transient holes to enhance the penetration, triggering wound repair mechanism spontaneously [103]. As expected, MN fails to cause severe erythema and post-inflammatory hyperpigmentation compared with laser for atrophic acne scars treatment [104]. In addition, MN can transport active cosmetic molecules into the skin directly for improving effectiveness and safety, creating microchannels without reaching nerve. Therefore, novel cosmetic patches loading retinyl retinoate and ascorbic acid were successfully produced for antiwrinkle without side effects such as allergies [105]. Nowadays, with the expansion of cosmetic market, commercial-scale manufacturing will witness the boom of MN products. This can be divided into two broad categories: patches and rollers. Penetrating MN patch into the skin at low velocity (e.g., slow insertion by hand) may cause low penetration effect, which is mainly due to the highly elastic skin [106,107]. To overcome this limitation, it is necessary to apply a high-velocity applicator to improve the

skin's instantaneous stiffness, thereby achieving complete penetration efficiency, which is inevitable to increase the cost of MN patch [106]. MN rollers, applied extensively in cosmetic field, were prepared from soft polymer films by inclined rotational UV lithography [108,109]. Currently, it exists MN presentation for cosmetics applications, for example, for the treatment of acne, pigmentation, scars, and wrinkles, as well as to improve skin tone [109].

HA is a major component of the extracellular matrix and is found in the skin, cartilage, bone, and many other tissues. It is commercially used in wound dressing products and has been widely studied for MN fabrication [110]. In fact, Microhyala is a HA-based MN array that is marketed for cosmetic use. HA-MN dissolves rapidly in interstitial fluid and is degraded within the body by free radicals found in the extracellular matrix and lysosomal enzymes [111]. On the other hand, Kim et al. [112] developed a HA-based dissolvable MN patch for the intradermal delivery of ascorbic acid and retinyl retinoate. Kumar et al. [113] showed an enhancement of local delivery of eflornithine (used to reduce facial hirsutism) in vitro and in vivo using a solid MN. Furthermore, MN technology was able to treat two patients suffering from alopecia areata disease; as a result, these patients experienced hair growth after treatment [114].

In another example of application, Yang et al. [115] evaluated skin restoration and wrinkle improvement using 5×5 arrays of dissolving MN containing horse oil (HOS) and/or adenosine (AD). The study showed that the HOS-AD-MN significantly improved skin elasticity, hydration, skin density, and wrinkles compared with the AD MN, without any side effects. In the same way, Kang et al. [116] evaluated skin parameters such as wrinkles, dermal density, elasticity, and hydration following combinatorial application of dissolving MN and cream with AD. They used 7×7 arrays of dissolving MN in this study. The combination therapy showed statistically significant efficacy in improving the average wrinkle depth, skin density, elasticity, and hydration. No adverse effects on the skin were observed during the trial period.

In one recent work, Avcil et al. [117] studied restoration of skin properties including hydration, wrinkle reduction, density, and thickness using HA-based MN containing bioactive peptides in 20 volunteers aged 40–71 years. The MN showed excellent resistance and effectiveness; no primary or cumulative skin reactions were reported in any of the subjects. Fine lines/wrinkles were noticeably reduced by 25.8%. Skin hydration measurements demonstrated a 15.4% improvement. Dermal skin density and

thickness increased by 14.2% and 12.9%, respectively. In addition to these studies, clinical trials over the past few years have demonstrated the application of MN beyond the cosmetic field, including chemical keratos, pigment disorders, hyperhidrosis, and striae [118].

As we have been reviewing, MN use in cosmetics is gaining importance, especially to improve the skin appearance and to treat skin blemishes and scars. An attempt to deliver some cosmetic active ingredients such as ascorbic acid, eflornithine, and retinyl retinoate was made using the MN approach [51]. In this case, melanin was incorporated into phosphatidylcholine liposomes (nanoliposomes), which showed increased solubility in lipids. Amount of pigment that reached deep near the hair structures was found to be more on application by an e-roller [119]. Enhanced delivery of melanostatin, rigin, and palmitoyl-pentapeptide was also investigated using MN [108].

10 Enhancing the action of hormones using NP-modified MN

Diabetes is a chronic disease, and diabetes-associated complications affect more than 425 million people all over the world [14]. Current treatment of diabetes depends on multiple daily injections of exogenous insulin to continuously regulate blood glucose levels. However, frequent insulin injections can lead to long-term complications and poor compliance, and insulin overdose can lead to severe shock or even death [120]. Therefore, there is a pressing need for a painless, noninvasive, and self-administration method that can be used repeatedly. More importantly, the dose should be adjusted according to the actual need. Moreover, as a transdermal drug delivery system, MN can avoid gastrointestinal irritation and first-pass effects by oral delivery [42]. Additionally, the gastrointestinal tract is an important active site of metformin hydrochloride, while metformin can cause gastrointestinal problems, including stomach pain, vomiting, and other side effects. Therefore, Migdadi et al. [121] designed hydrogel-based MN to allow sustained delivery of metformin through the transdermal drug delivery system.

In 2009, an MN was first used to deliver insulin to human subjects. Gupta et al. [122] proved that hollow MN could availably deliver burst insulin to patients who had type I diabetes in a minimally invasive way. Lee et al. [123] fabricated an MN by reverse drawing lithography. Additionally, they assembled this MN with a 1 mL disposable syringe. They found that after the

application of an MN and pen needle injection on 15 patients with type II diabetes and 25 healthy adults, the MN could manage blood glucose more steadily, similar to the traditional hypodermic needle.

Concerning MN technology, Resnik et al. [124] designed and manufactured hollow silicone MN to microinject insulin. In vivo results showed a successful infusion of fast-acting insulin determined by monitoring the glucose levels in plasma. Specifically, MN-based delivery granted a less significant drop in glucose levels, but a significant increase in serum insulin levels (40%–50% of increase), due to a more effective delivery of exogenous insulin. Lee et al. [125] created dissolving MN composed of gelatin and CMC in a two-step casting and centrifugation process to concentrate the insulin in the needle tip and improve the transdermal delivery efficiency. Ex vivo results showed that 50% of the insulin was released and penetrated the skin after 1 h, with cumulative permeation reaching 80% of the initial dose after 5 h. In vivo results proved a bioavailability of insulin from MN of 95.6% and 85.7%, respectively.

Another example for dissolving MN in this area is the work performed by Chen et al. [126], who prepared MN with poly-c-glutamic acid (c-PGA) and PVA/polyvinyl pyrrolidone (PVP) as supporting structures, to be dissolved in 4 min upon skin insertion. The results, obtained in a rat model, indicated that the hypoglycemic effect achieved with insulin loaded MN was comparable with the one observed with a conventional subcutaneous insulin injection. The bioavailability of insulin was in the range of 90%–97%. Moreover, no significant differences were observed in the plasmatic insulin concentration profiles between serial administrations, demonstrating the stability and accuracy of the MN. Tong et al. [127] loaded PVP/PVA MN with glucose and H_2O_2-responsive polymeric vesicles containing insulin. This device is specially designed to deliver insulin under hyperglycemic conditions. Vesicles were made by a self-assembling method and formulated with three polymers: poly(ethylene glycol), poly(phenylboronic acid), and poly(phenyl boronic acid pinacol ester). Poly(phenylboronic acid) is a glucose-sensitive polymer, and poly (phenyl boronic acid pinacol ester) is a H_2O_2-sensitive polymer. These polymers are hydrolyzed at hyperglycemic states and in the presence of a H_2O_2 stimulus, respectively. Polymeric vesicle contents were coated with glucose oxidase, which is an enzyme that converts glucose into glucuronic acid and then into H_2O_2 in the presence of oxygen. Controlled release of the drug from the polymeric vesicles was confirmed by a release NP study at different glucose and H_2O_2 concentrations (200 or 400 mg/dL). The presence of glucose oxidase in the particles

provided a faster insulin release since the breakage of the bonds in the poly (phenyl boronic acid pinacol ester) is prompted by the generation of H_2O_2. Then, this group tested the system in a type-2 diabetic animal model. Glucose-sensitive patches were administered to diabetic rats, and their glucose blood levels were monitored. MNs loaded with polymeric responsive vesicles were shown as a good alternative to subcutaneous insulin injection, since they achieved an effective and longer-lasting hypoglycemic effect. The system provided a decrease in the baseline glucose level from 500 to 110 mg/dL in 4 h and a gradual recovery to the initial glucose levels during the following 8 h. In contrast, the subcutaneous injection caused a decrease in the baseline glucose levels down to 80 mg/dL in 2 h and a faster recovery to basal levels (in the next 5 h). The decrease in glucose levels (maximum effect) with subcutaneous injections was faster due to the inherent behavior of the patch during release. However, normoglycemic levels (under 200 mg/dL) were maintained for 4 h, in comparison with subcutaneous injection, in which they were maintained for only 2.5 h.

A similar outcome was achieved using other NPs and patches. Yu et al. [128] designed MN containing nanovesicles loaded with insulin and glucose oxidase. Glucose-responsive nanovesicles were self-assembled from hypoxia-sensitive HA conjugated with 2-nitroimidazole. As aforementioned, the local hypoxic microenvironment is caused by the enzymatic oxidation of glucose in H_2O_2 at the hyperglycemic state and is responsible for the dissociation of the vesicles and the patch, allowing the subsequent release of insulin. Similar results were observed using a type-1 diabetic mice model, in which an effective and controlled regulation of blood glucose was achieved. Particularly, a glucose tolerance test was conducted 1 h after administration of the MN. The control healthy mice exhibited a quick increase in blood glucose level upon an intraperitoneal glucose injection, followed by a gradual decrease to normoglycemia. The diabetic mice treated with MN, which contained insulin and glucose oxidase-loaded particles, showed a delayed increase in blood glucose after glucose injection and then, a rapid decline to a normal state within 30 min. However, the glycemia of the mice receiving insulin-loaded MN did not decline in 120 min, confirming that the MN, which contained insulin and glucose oxidase-loaded particles, had significantly faster responsivity toward the glucose challenge. In another study, Ye et al. [129] investigated MN integrated with pancreatic β-cell capsules, which sense the blood glucose levels and secrete the insulin. But the patch was not found to function effectively. Thus, MN matrix containing synthetic glucose signal amplifiers was developed, which consisted of

nanovesicles containing glucose oxidase, α-amylase, and glucoamylase enzymes. These amplifiers showed the secretion of insulin from the β-cell capsules.

In this way, Chen et al. [130] reported a smart and pH-responsive exendin-4 (Ex4) delivery MN patch based on alginate for type 2 diabetes therapy. This patch loaded with dual mineralized peptide/protein NPs could separately control the release of Ex4 to avoid rapid loss without multiple daily injections. To construct long-term glucose responsiveness, copper phosphate mineralized particles containing glucose oxidase played a potent role in converting glucose to H^+ signals under hyperglycemic conditions, while calcium phosphate mineralized particles encapsulating Ex4 as a pH-sensitive biomaterial can be dissolved to release Ex4 under acidic condition. An alginate-based MN patch would be an effective candidate to realize facile administration for type 2 diabetes patients [56].

Following this approach of the applications of MN for hormone delivery, the MN can also be used to deliver a large number of macromolecules in a controllable manner, for example, growth hormones (rhGH) and parathyroid hormone. The first studies for rhGH delivery stand out in the work reported by Ito et al. [131]. In this design, self-dissolving micropiles (SDMPs) were tested as a percutaneous delivery system in rats using dextran as a base. After mixing dextran solution with rhGH, SDMPs were prepared by pulling with polypropylene tips. To evaluate the bioavailability of rhGH percutaneously administered by SDMP, an absorption experiment was performed in rats. RhGH SDMPs were inserted into the rats' skin, 200 μg/kg, and plasma rhGH levels were measured by an ELISA method. Peak plasma rhGH level, 132.8 ± 11.8 ng/mL, appeared at 0.8 ± 0.2 h. By comparing the plasma rhGH levels versus time profiles after the administration of SDMP and intravenous injection of rhGH solution, 5 μg/kg, bioavailability of rhGH from SDMP was calculated to be 87.5%.

Based on the previously described and as a new design, a dissolvable MN patch was used to deliver rhGH for transdermal delivery to hairless rat skin [132]. In this study, the dissolving MN was composed of carboxymethylcellulose and trehalose using an aqueous, moderate-temperature process that maintained complete rhGH activity after encapsulation and retained most activity after storage for up to 15 months at room temperature and humidity. After manual insertion into the skin of hairless rats, rhGH pharmacokinetics were similar to conventional subcutaneous injection. After patch removal, the MN had almost completely dissolved, leaving behind only blunt stubs. The dissolving MN patch was well tolerated, causing only slight, transient

erythema. This study suggests that a dissolving MN patch can deliver rhGH and other biopharmaceuticals in a manner suitable for self-administration without sharp biohazardous waste.

With the objective to optimize the permeation of rhGH, MN application and iontophoresis are strategies that can be used to overcome this barrier. In the work reported by Noh et al. [133], methylene blue staining, stereomicroscopy, and scanning electron microscope imaging were used to characterize the produced microchannels. To optimize the iontophoresis protocol, the effects of molecular charge and current density on transdermal delivery were evaluated in an in vitro permeation study using excised rat skin tissues. Using the optimized iontophoresis protocol, the combination effects of iontophoretic delivery through microchannels were evaluated in three different experimental designs. The flux obtained with anodal iontophoresis in citrate buffer was approximately 10-fold higher than that with cathodal iontophoresis in phosphate-buffered saline. Flux also increased with current density in anodal iontophoresis. The combination of iontophoresis and MN application produced higher flux than single application. These results suggest that anodal iontophoresis with higher current density enhances the permeation of macromolecules through microchannels created by MN.

Finally, the results of clinical study conducted for parathyroid hormone coated MN demonstrated three times shorter T_{max} and two times shorter apparent $T_{1/2}$ compared with conventional injection therapy [134]. These studies indicated that MN can be utilized efficiently for the hormonal therapy. Further, these can also be modified for sustained action with the use of suitable polymers [135].

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CHAPTER 3

Types of microneedles for drug delivery

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1 Limitations of classical routes for drug administration

Every year, health science researchers focus on the design of devices that enhance the delivery of drugs to improve the quality of life of patients. Not only the native physicochemical properties of many drugs, such as hydrophobicity and low permeability, make that conventional therapies have a limited efficacy, but also the route of administration and the mechanism underlying its delivery in the tissue are constant problems that must be overcome in order to have increasingly effective therapies. For these reasons, it is crucial to develop optimal methods for drug delivery in accordance with the properties of the drug.

There are different routes for drug delivery into the human body [1], such as oral, parenteral, inhalation, transdermal, etc. The oral route is the oldest one and the most accepted by patients. This route offers many advantages since the patient can self-administrate the drug with acceptable ease. Furthermore, oral solid pharmaceutical forms (tablets, capsules) generally have good physicochemical stability and easy dosing. However, these formulations also face certain obstacles that may impair the bioavailability of the drug such as a hepatic first pass effect, formation of nonabsorbable complexes with several ions, hydrolysis by gastric pH, gastric and intestinal motility, and malabsorption due to diseases or surgeries [2–5]. In addition, during long-term medications, the oral route has a considerable side effect because it impacts vital organs such as the liver and kidneys. Beside all the aforementioned drawbacks of oral administration, the nonadherence of the therapeutic guidelines by the

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patients is very common and makes it harder to achieve drug plasma levels within the therapeutic range [6].

For all these reasons, the most used alternative to the oral route is the parenteral administration (intramuscular, subcutaneous, and intravenous pathway); these routes allow, for instance, the introduction of hydrophobic drugs into the human body [7]. The parenteral drug delivery is classified as a rapid delivery method and is considered a powerful tool of drug delivery in emergency situations [8]. Usually, the parenteral administration introduces drugs into the body using a needle, which overcomes the obstacles of the aforementioned drug absorption mechanisms for oral administration. However, parenteral administration comes with other limitations, for example, the need of aseptic materials and techniques, generation of pain during the administration, and other medical complications such as thrombus formation or hypersensitivity reactions and the need of trained personnel. All these requirements mean that this alternative is not preferred by many patients [9]. The disadvantages and limitations of oral and parental routes triggered the research toward other administration sites. In this sense, rectal mucosae, buccal epithelia, or sublingual membrane were explored and demonstrated to be unpredictable, limited, or erratic [10]. Through the years, numerous efforts have succeeded in improving the pharmacological effect by applying the drug in a localized region of the body, thus reducing the toxic effect in the surrounding tissue or in other nontarget tissues [11]. Nowadays, the strategy known as targeting has received increasing attention. Targeting enables the drug to directly reach the target organ or tissue, thereby reducing the systemic exposure and consequently, in many cases, reducing or avoiding toxic side effects [12]. In particular, the respiratory, ophthalmic, and cutaneous routes can easily release drugs directly into the corresponding target tissues [13,14]. For example, the inhalation route is designed to deliver the drug directly to the lungs. In principle, this route is painless, comfortable, and is designed to treat diseases related to the respiratory system. Moreover, certain drugs are shown to be efficacious in crossing the air-blood barrier [15]. As we have been describing another drug administration routes, the inhalation route exhibits certain disadvantages and risks associated with overdosing through self-administration by the patients requiring multiple doses each day [16]. For these reasons, lots of attempts have been made to further improve the accuracy in dose delivery and potency of drugs, with the aim to reduce their risk in patients by identifying natural or synthetic material matrices with tunable release kinetics [17–19].

2 Transdermal drug delivery

Another option, not least to the aforementioned routes, falls on the administration through the skin, which is another powerful possibility for drug administration that can be used as an alternative to oral drug delivery in neonates and geriatric patients having difficulty in swallowing medications [20,21]. The skin has a large available surface, with an area of approximately 20,000 cm^2 in adults. For this reason, one of the skin main functions is to prevent the entry of exogenous substances into the body [22]; therefore, the skin per se acts as a barrier for molecules absorption. The skin gets this protection by the lipophilicity and the great cohesion between the cells of the most external layer of the skin (*stratum corneum* (SC)) [23]. Research on cutaneous permeability has shown that, when using a suitable formulation, the skin may be also used for systemic administration of drugs, a process called transdermal drug delivery [24]. This happens because the dermis (one of the deeper layers of the skin (Fig. 1) is rich in blood microcirculation, and when the drug reaches this layer is absorbed [25].

In this way, one drug can be transdermally administrated formulated as gel, cream, ointment, or patch, as shown in Fig. 2 [26]. This approach allows the controlled drug release in a minimally invasive manner compared to the bolus drug delivery via hypodermic needles [22]. The transdermal route is used to slowly transport drugs from the skin surface into the body [26,27]. This way is generally well accepted by patients since, for some indications, it is more convenient and efficient to "put on a patch" than to "pop a pill" [28]. Moreover, it has the advantage of not affecting directly vital organs when delivering potent drugs and provides an alternative mechanism for sustained delivery [29–31].

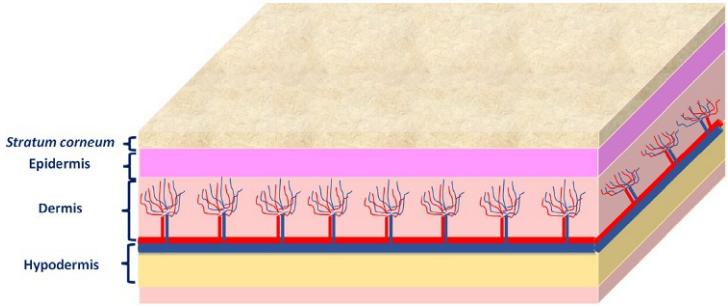

Fig. 1 Layers of the skin.

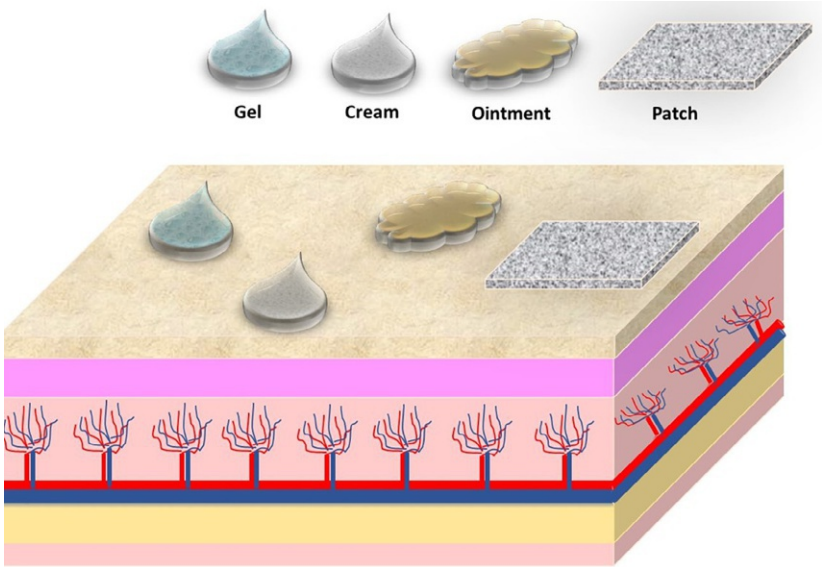

Fig. 2 Classical formulations administered transdermally.

In a transdermal administration, the main process of drug delivery through the skin is passive diffusion, either by intracellular route (the drug is dissolved in the lipidic matrix where the corneocytes are embedded) or by transcellular route (the drug crosses through the cells) [32]. As an any passive process, it follows the Fick's laws, pointing out the importance of the drug concentration in the vehicle and the partition coefficient between vehicle and membrane, among other parameters that condition drug absorption [33]. The diffusion itself depends on both, the integrity of the skin and the physicochemical characteristics of the molecules, which must have a low degree of ionization [34], an intermediate distribution coefficient (Log $P \approx 1–3$), and a molecular weight of less than 500 Da [35].

Although the blood circulation is close to the skin surface, the delivery of hydrophilic drugs, charged molecules, peptides, proteins, and nucleic acids represents a challenge, since the skin is designed to prevent the entry of foreign substances [22,23]. The complexity of the drug penetration process into the skin depends on both the lipid matrix structure and mechanical properties of the skin, i.e., whose parts are influenced by environmental conditions (humidity and temperature) [32].

3 Microneedle device (MND) for transdermal drug delivery

Nowadays, diverse strategies have been developed to overcome biological barriers, especially the skin, using noninvasive or minimally invasive techniques. As mentioned in the previous section, SC is a barrier that considerably limits the transdermal delivery of larger molecules such as proteins and therapeutic genes. To increase the permeability of the SC, techniques such as ultrasound, iontophoresis, electroporation, thermal ablation, and microdermabrasion have been developed [36]. However, most of these techniques have not succeeded in passing the preclinical phase of testing due to the risk of skin irritation, which is unacceptable in a clinical setting [37]. Permeation enhancers and prodrug approaches have also been explored extensively for transdermal administration with limited success [38].

To overcome the challenges associated with current technologies, MNDs are currently of significant interest in both transdermal drug delivery and transdermal diagnostics. MNDs are minimally invasive devices consisting of numerous micron-sized projections arranged on a baseplate [36]. The MNDs provide a physical enhancer since they create a physical disruption in the SC, forming temporary aqueous micropores through which drugs can diffuse to the dermal microcirculation [39]. However, with an average height of 400–900 μm, MNDs are short enough to avoid stimulation of dermal nerves and do not induce bleeding [36]. Thus, it is possible to deliver hydrophilic drugs through this method or one may utilize sweat glands as an alternate mechanism. So, these micron-sized sharp needles can directly deliver molecules across the skin, the cornea or back of the eye, or even the gut membrane [40,41].

The concept of miniature needles for drug delivery came about in the 1960s, and it was patented by Alza Corporation in 1971 [42]. That device contained tiny protrusions and a drug reservoir for biological agent delivery by diffusion or pressure. This MNDs concept was experimentally tested almost 30 years later, with the extensive development of microfabrication manufacturing technology.

From the first published scientific paper on drug delivery using MND [43], interest in improving MNDs has grown continuously, along with the number of clinical trials and approved products based on this principle [44]. The MNDs can be broadly classified based on their shape (conical, pyramidal, obelisk, etc.), material of fabrication (metal, glass, silicon, polymer, etc.), or technique of drug loading and delivery (hollow, solid, coated, etc.) [45].

Broadly, drug delivery using different types of MNDs has been classified into five different approaches [46]. In this way, there are several ways to classify MNDs. Some authors divide them into solid and hollow MNDs, including coated, uncoated, and dissolving MNDs in the category of solid ones [47]. Others divide them according to the production method into "in-plane MNDs" (microneedle shafts oriented parallel to the base substrate) and "out-of-plane MNDs" (microneedle shafts bent at 90° to the base substrate) [48]. The most common categorization is into five types, which will be revised in detail in the next section [49].

4 Types of MNDs for drug delivery

As mentioned earlier, a variety of materials such as silicon, stainless steel, sugar, and polymers have been used to fabricate solid, coated, hollow, or dissolvable MND. Each type of the MND has its unique characteristics, advantages, disadvantages, applications, and material type [50]. Diverse types of MNDs have been investigated since the success for drug delivery is determined by critical issues, mainly concerning the design of the MNDs (shape, size, geometry, manufacturing materials, and processes) and the type of delivered active substance. Once the MND is defined, an additional strategy can be selected for the drug delivery. These different approaches can be classified into the following: "poke and patch," "coat and poke," "poke and flow," and "poke and release" [51].

4.1 Solid MNDs

4.1.1 Solid MNDs for the "poke and patch" approach

Starting with the "poke and patch" approach that consists in the use of solid MND to perforate the skin, this methodology is focused on the creation microchannels that reach the deepest layers of the epidermis. This method significantly improves the passive transport of drugs through the skin, since it allows the disruption of the SC [52,53]. In addition, in comparison to intramuscular delivery, the solid MND is suitable for delivery of vaccines as it lasts longer and possesses a more robust antibody response [50].

So, the approach of using a solid MND presents two steps: First, the MNDs are used to pierce the epidermis and are subsequently removed; and second, the drug is applied in a conventional dosage form (solution, cream, or patch), which works as an external drug reservoir (see Fig. 3) [54]. In principle, its simplicity from the technological point of view makes it highly attractive, especially for its easy application in a clinical setting.

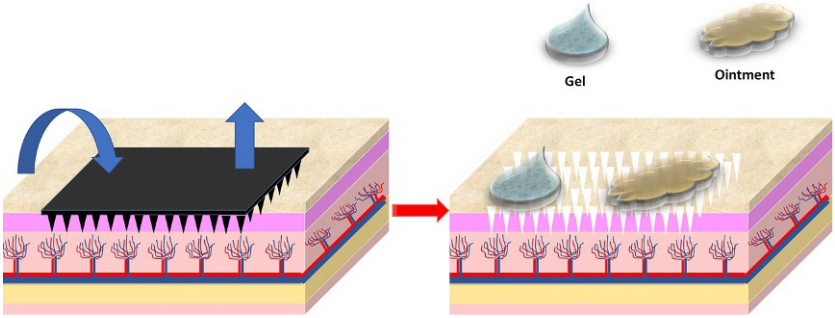

Fig. 3 Solid MNDs for the "poke and patch" approach.

However, this technique is not exempt of controversy and presents several disadvantages. One of the main drawbacks is that the micropores remain open only for a limited period of time, potentially stopping the delivery of the active substance prematurely [50]. It has been reported that all MND-treated sites recovered their barrier properties within 2 h [53]. Nevertheless, this period can be extended up to 3 days under occlusive conditions by using formulations like patches or tapes [55], although the risk of infection increases considerably in these conditions [56].

Solid MNDs are easy to manufacture and have superior mechanical properties and sharper tips when compared to hollow MNDs [50]. Moreover, the solid MND can be fabricated from different materials such as silicon, metals, and polymer [44].

4.1.2 Coated solid MNDs for the "coat and poke" approach

Another approach with solid MNDs is the "coat and poke" approach; this strategy requires the coating of the solid MND surface with the adequate biological agent (i.e. loaded with drug, protein, peptide, or deoxyribonucleic acid (DNA)) [46,57]. This approach allows drug diffusion from the coating surface to the deeper epidermal layers after MND insertion, as shown in Fig. 4 [58]. The success of delivering drug using a coated MND depends on the ability to reliably coat a controlled drug layer onto MNDs [59]. Although this approach allows the rapid delivery of the therapeutic agent, a limited drug amount can be applied on the base and shaft of the MND (usually less than 1 mg) [59]. In addition, the coating's thickness can decrease the sharpness of the MNDs and affect their ability to perforate the skin [60]. Despite this, coated MNDs have shown great efficiency in vaccination, since the antigen dose needed to trigger an immune response is usually in the range of nano or micrograms [61].

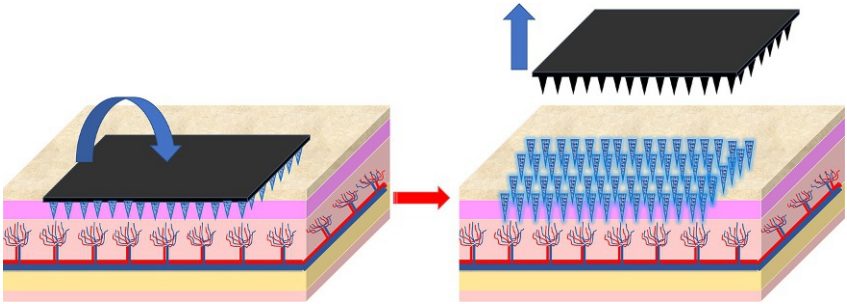

Fig. 4 Coated solid MNDs for the "coat and poke" approach.

A more recent focus consists on the integration of these MNDs, as a part of a closed-loop system of a smart patch, which allows the controlled drug delivery based on feedback from the analysis of body fluids [46,62]. With these systems, the incorporation of the lab-on-a-chip technology will enable the detection of broadly employed biomarkers not only in hospitals but also in clinical laboratories [46].

4.2 Hollow MND

4.2.1 Hollow MNDs for the "poke and flow" approach

The "poke and flow" approach was conceived to introduce a drug solution into the skin mimicking hypodermic injections while overcoming their limitations [63,64]. The hollow MND consists of a design with a hollow/empty core/chamber (5–70 μm wide) in which the drug fluid is injected/stored (see Fig. 5) [31]. Compared to the solid MND, the hollow MND can handle a large dose/amount of drug solution (typically, 10–100 μL/min) [26]. A hollow MND has also the ability to deliver a drug into the viable epidermis or dermis, which is suitable for high-molecular-weight compounds [65].

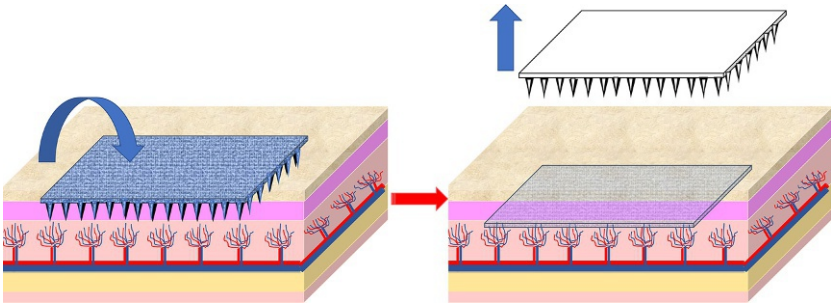

Fig. 5 Hollow MNDs for the "poke and flow" approach.

Additionally, it controls the drug release over time, which makes it suitable for use with liquid vaccine formulations and insulin [66].

Hollow MNDs are an active drug delivery system that form a conduit for drug diffusion into the dermis, which, in general, are based on a nonpressurized drug reservoir. In this way, both material formulation and fabrication parameters of hollow MND can be leveraged to enable tunable release kinetics [50]. So, higher concentration drugs can result in burst release drug profiles, whereas matrix-loaded drugs can enable a steady-state drug release, lasting days to weeks, depending on the application intent [67].

Another investigated strategy involves the control of the release of bioactive agents which can be achieved by incorporating a microfluidic chip [68,69] or micropump [70] into an array of MNDs [48,71]. The integration of the drug reservoir with a heater delivers the drug solution into the skin by spreading the liquid or creating bubbles. By pressing the flexible reservoir, the drug solution is released into the skin [47,71,72]. Although this therapeutic strategy requires the liquid drug formulation, no reformulation is necessary [46]. However, a challenge is to deliver a dry formulation, generally used to improve the drug stability and convenience of the patch-based application, without reconstitution [44]. The main limitations are the possibility of clogging the needle tip in the tissue and the resistance to flow due to the density of the compressed skin tissue around the MND tip. Therefore, to overcome this disadvantage, a side opening with off-centered holes is designed [46]. Another way is the gradual insertion of the needle [54].

Finally, this type of MND has received less attention compared to the solid MND since the hollow MND is relatively weaker and requires intensive care in terms of needle design and insertion method [73]. Currently, there are two examples of FDA-approved hollow MND (BD Soluvia and the Nanopatch) in the market using this approach to deliver influenza vaccine intradermally [74]. Furthermore, the hollow MNDs suffer from technical difficulties such as leakage and clogging during the injection process, which implies an additional obstacle [50].

4.3 Dissolving MNDs for improvement of the "poke and patch" approach

Another design consists in the use of dissolving MNDs that are built through a soluble matrix containing biocompatible polymers or sugars [36,46]. MND tips dissolve after contact with the interstitial fluid, followed by therapeutic agent release ("poke and release" approach) (see Fig. 6), so the application involves only a single step since the MND is not to be

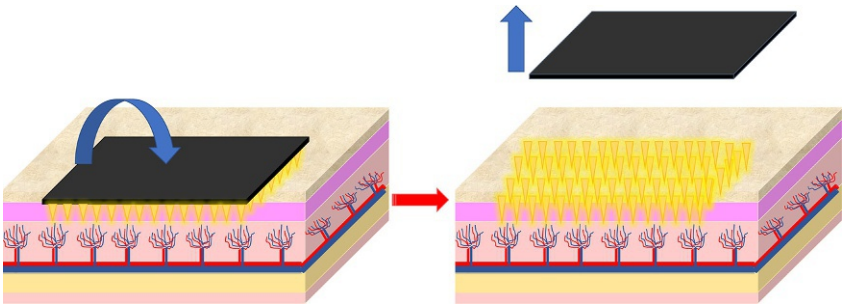

Fig. 6 Dissolving MNDs for improvement of the "poke and patch" approach.

removed out after insertion as in other cases. Using this design, the release kinetics of the active substance depends on the degree of dissolution of the constituent polymers, so that it is possible to control drug delivery by adjusting the polymer composition or by modifying the manufacturing process [54].

Nowadays, there is a growing interest in dissolving MNDs made of biodegradable materials, as they enable drug delivery without creating sharp, biocontaminated, and nondegradable waste [44]. Moreover, in the case of the production of MNDs from semisynthetic and synthetic polymers and sugars, the manufacturing costs are significantly lower [75]. In this regard, another improvement seen in this approach, in comparison with the "poke and patch" approach, is that dissolving MND can maintain controlled drug release over a longer period of time, thereby controlling the dissolution rate of the formulation used as the MND matrix. Another advantage is the reduction of the drug administration process to one step, since the MNDs are able to pierce the skin and are kept inserted until complete dissolution [76,77].

For instance, Ling and Chen presented a dissolving MND patch consisting of starch and gelatin, and they tested insulin as a therapeutic model molecule [78]. An in vitro test showed that these MNDs released almost all the insulin content within 5 min. Furthermore, they were mechanically strong enough to enable the bioactive molecules to be stably encapsulated [46].

For these designs, the main disadvantage is the rapid deposition of polymers in the skin, which is undesirable if the design is for long-term use [54]. Degradable MNDs, a subcategory of dissolving MNDs, may deliver a wide range of hydrophilic agents, including caffeine, lidocaine, metronidazole, ibuprofen, as well as several biopharmaceutical molecules (low-molecular-weight heparin, leuprolide acetate, erythropoietin, and human growth hormone) [78]. Finally, other drawbacks include a limited drug loading and a potentially lower ability to perforate the SC.

4.4 Hydrogel-based MNDs for the "poke and release" approach

Following the "poke-and-release" approach, the mechanism of hydrogel-based MND drug delivery is the same as with dissolvable MND: insert into the skin, release the drugs, and there is no need to discard the needle (see Fig. 7) [79]. The polymers selected for these designs bear a hydrophilic structure, which makes them capable of taking up a large amount of water into their three-dimensional polymeric network [80]. These polymers swell when inserted into the skin due to the presence of the interstitial fluid. This leads to the formation of channels between the capillary circulation and the drug patch. Before needling, these MNDs are just used to disrupt the skin barrier. So, on swelling, they behave as a rate of drug delivery controlling membrane. The hydrogel-based MNDs have flexibility in size and shape, are easy to sterilize, and can be intactly removed from the skin [56].

So, these systems are manufactured from aqueous mixtures of specific polymeric materials. In this regard, Lee et al. made hydrogel MNDs from ultra-low viscosity carboxymethylcellulose (CMC) and amylopectin [81]. After the incorporation of sulforhodamine, bovine serum albumin, or lysozyme, MNDs were safely dissolved in the skin, therefore allowing bolus or sustained release delivery. On the other hand, Garland et al. showed that drug delivery could be adjusted by modulating the density of the hydrogel matrix [82]. Importantly, after the removal of the hydrogel MND, the skin was intact, leaving no polymer residues. Hydrogel MNDs have softened enough to prevent reapplication, thus reducing the risk of transmitting infections.

In general, using hydrogel-based MNDs allows the release of less potent drugs contained in an attached patch-type drug reservoir [83,84]. For example, Migdadi et al. studied hydrogel-forming MNDs to administer metformin transdermally and decrease the gastrointestinal side effects associated with the oral delivery. The results demonstrated the improved permeation and bioavailability of the drug with designed MND [44].

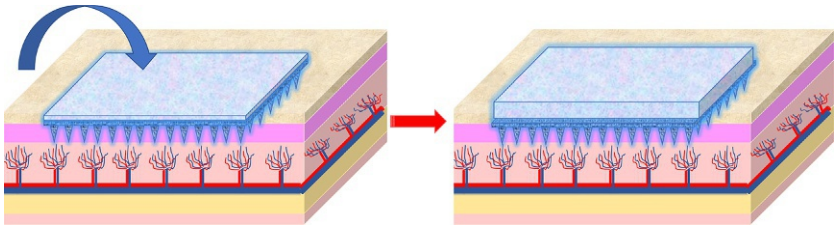

Fig. 7 Hydrogel-based MNDs for the "poke and release" approach.

Although dissolving or degradable MNDs could carry higher concentrations of the drug, the matrix makes up a large portion of the needles, which dissolve or degrade in the skin, making them unsuitable for everyday use. Retaining the matrix in the skin may cause low compliance in patients and potential side effects if the drugs are administered daily or at short intervals. The most suitable active ingredients, in this case, are vaccines because they are used only once (or a few times), so the amount of matrix deposited in the skin will be more acceptable [46]. Therefore, the most promising type of MND is one made of hydrogel that does not dissolve nor degrade in the skin but has a controlled or continuous release of active substances [49].

5 Background of materials for the construction of MNDs

After reviewing the different parameters that an MND must meet, the material of fabrication of MNDs has emerged as a critical factor in their manufacture and usage. In fact, different shapes and designs of MNDs require specific properties of the material for the preparation [74]. In the early stages of MND development, MNDs were exclusively prepared with silicon [45]. However, their main disadvantage was the breakage of the silicon MND due to their brittle nature. These solid MNDs were prepared using lithography; sooner afterward, hollow glass MNDs were prepared using glass-blowing and pulling. These MNDs were tested for intradermal delivery of influenza. On the other hand, metal MNDs have sufficient mechanical strength to penetrate the skin, but their disadvantage is that they generate potential biological waste [26,27]. Interestingly, nitinol is used in vascular surgery due to its advantages in terms of elasticity, shape-memory capability, and biocompatibility [85]. In addition, polymeric MNDs have better solubility and usage in case of the tip breaking [50,86]. Water-soluble polymers [27,86–88] and engineering plastics such as CMC, poly (gly*co*lic acid) (PGA), polylactic-co-glycolic acid (PLGA), poly (vinyl alcohol) (PVA), poly (vinylpyrrolidone) (PVP), polylactic acid (PLA), chondroitin sulfate, and polycarbonate are employed for MND production, whereas dissolving MNDs are composed of sugars such as maltose [46,89], dextran [90], or galactose [44,91–93].

So, a combination of different types of materials has been utilized for biomedical applications in drug delivery, tissue engineering, and biomedical implants [94–96]. Therefore, it is necessary to select the most suitable materials for MND production based on the following criteria [97]: gentle manufacturing without damaging sensitive and unstable molecules, controlled or immediate drug release, and sufficient mechanical strength for skin penetration [46].

5.1 Materials for the construction of MNDs

5.1.1 Silicon

As aforementioned, the first MND was made from silicon in the 1990s [45]. Silicon is anisotropic in nature and has a crystalline structure. Its properties depend on the alignment in the crystal lattice, which shows different elastic moduli (50 to 180 GPa) [54,76,98]. Its flexible nature allows to produce needles of different sizes and shapes. Its attractive physical properties make it a versatile material. Silicon substrates can be precisely manufactured and are suitable for batch production [31]. The cost of silicon and its time-consuming complex fabrication process limits its use in MND. In addition, there are some biocompatibility issues since silicon is brittle and some parts may break and remain in the skin, thus causing some health issues [54].

5.1.2 Metals

The main metals used for MND elaboration are stainless-steel and titanium. Palladium, nickel, and palladium-cobalt alloys are also employed [99], since they have good mechanical properties and good biocompatibility. Metals are strong enough to avoid breaking, thus being more suitable, compared to silicon, for MND production. The first metal used in the production of MND was stainless steel [100], but titanium is also a good alternative [54].

5.1.3 Ceramic

Ceramics are solid materials composed of inorganic compounds such as metals, nonmetals, or metalloids. Ceramics are solid, possess thermal and electrical insulator properties, and have a brittle nature [74]. Biocompatible ceramics possess higher mechanical strength and higher temperature and moisture stability than most polymers. The porosity of ceramics can be easily tailored during their production. This property of ceramics in addition to electrostatic interaction between the ceramic surface and permeants can be exploited to enhance transdermal permeation of molecules [101]. Most common ceramics used as biocompatible materials are those bearing aluminum and oxygen (alumina, Al_2O_3), calcium and oxygen (CaO), and silicon and nitrogen (silicon nitride, Si_3N_4). Alumina and zirconia (ZrO_2) are commonly used to fabricate ceramic MNDs [102]. Alumina is biologically inert, stable at high temperatures but brittle under tensile stress [103]. Since alumina is sintered at higher temperatures, the loading of thermo-labile molecules could be a challenge. Zirconia offers higher toughness and strength than alumina but has poor wear characteristics. Calcium compounds, calcium sulfate,

and hydroxyapatite are also attractive materials for fabrication of MNDs [104,105].

Ceramic MNDs are typically prepared using micromolding of a ceramic slurry followed by sintering at high temperatures. The slurry parameters can be adjusted to result in variations in ceramic morphology, porosity, and strength. Organically modified ceramic, Ormocer, has been used to prepare MNs. Ormocer is synthesized through a solution and gelation process (sol-gel process) from multi-functional urethane and thioether(meth)acrylate alkoxysilanes [106]. Ceramic MNs prepared using CaS and CaP were employed to study the release of loaded zolpidem tartrate into the skin [107]. In another study, nanoporous ceramic MNs made from alumina were utilized for transcutaneous vaccination [108].

5.1.4 Carbohydrates

Melts, solutions, or slurries of carbohydrates can easily form MNDs by micromolding in metal. These MNDs generally dissolve quickly by taking up interstitial fluid and releasing the entrapped drug molecule. Carbohydrates offer a cheap and safe alternative for metals and glass. However, carbohydrate needles possess lower tensile strength and need to be combined with other materials for increasing the strength. Maltose is the most commonly used sugar for preparation of MNDs. Maltose-based MNDs were studied for the transdermal permeation of nicardipine hydrochloride across rat skin. Maltose MNDs, $508.46 \pm 9.32\,\mu m$ long with a tip radius of $3\,\mu m$, were prepared by micromolding [74]. Pretreatment of the skin with maltose MND significantly improved the flux of nicardipine across the skin in comparison with untreated skin [109].

5.1.5 Polymers

Polymers offer a promising material alternative for the elaboration of MNDs. They have excellent biocompatibility, low toxicity, and low cost [50]. However, they also possess lower strength compared to silicon and metals [50]. Polymers are usually employed in the production of dissolvable and hydrogel-forming MND's arrays [67], solid, coated, and hollow MND arrays [54]. Various types of drugs have been applied to the skin using biodegradable MNDs [110]. Types of polymers used in the fabrication of MNs included poly (methyl methacrylate) (PMMA), polylactic acid (PLA), poly (carbonate), polystyrene, and SU-8 photoresist [31].

Another approach consists in the use of natural protein-based polymers such as collagen, gelatin, or zein which are approved for different

pharmaceutical usages. Collagen is largely biodegradable and biocompatible. However, a few reports indicate poor stability upon swelling in vivo, non-specific immune reactions, and tissue reactions [74]. Collagen swells upon contact with aqueous media but can only be digested by specific collagenases and pepsin-cleaving enzymes [111]. Gelatin, irreversibly hydrolyzed form of collagen, is a common constituent in food additives and soft capsules. Gelatin, in combination with other materials, is also studied for MND fabrication [102,103]. Due to the absence of aromatic groups (no Tyr and Trp, and low Phe), gelatin is not antigenic. Like collagen, gelatin also degrades after swelling by the action of specific peptide cleaving enzymes [74].

MNDs have evolved from solid or hollow structures to newer concepts employing the use of bifunctional materials. Surface modifications for better drug loading and release, combination of complex nanosystems with MNDs, and integrated sensing and control systems for responsive on-demand drug delivery are few examples. Advancements have been made in glucose measurement with algorithmic spatial control glucose measuring and a feedback mechanism to deliver insulin on-demand. MNDs have shown potential to be developed as minimally invasive continuous glucose monitoring systems.

5.1.6 Advanced materials and combinations

Transdermal delivery of micro and nanoparticles can be improved by MND assistance. The particles can be delivered through the conduits of hollow MNDs, coated onto solid MNDs or encapsulated within dissolving MNDs [112–114]. Delivery of other therapeutics, especially proteins and vaccines, can be enhanced by surface modifications of MNDs. pH modification using pyridine on the surface of silicon MNDs resulted in a better loading and release of coated ovalbumin in the skin [115]. Polyplex-based DNA vaccines were rapidly delivered using polycarbonate MND arrays coated with multiple polyelectrolyte layers of charge reversal pH-responsive copolymers [116].

6 Manufacturing methods for the construction of MNDs

Prior to selecting the methodology for the construction of an MND, the first step is to be sure about the approach that the MND will have. In this regard, the drug type and dose, desirable pharmacokinetics/pharmacodynamics, and targets for their use are considered. As soon as the most optimized MND design and materials are determined, the manufacturing method for MNDs varies, depending on the design or material. For example, focusing on the economic aspect, a method such as solvent casting, which is easy to set

up, is used. In contrast, if the focus is on the accuracy, precision, and reproducibility of needle production, production of metal or silicon MNDs based on microelectromechanical systems (MEMS) technology can be considered [117]. In the following sections, various methods reported till date for MND manufacture are summarized.

6.1 Laser-mediated fabrication techniques

6.1.1 Laser cutting

Laser cutting is primarily used for manufacturing metal or polymer MND; in fact, the most used material is stainless steel [118,119]. Arrays of solid MNDs are produced by cutting stainless steel or titanium sheets in the shape of MNDs with an infrared laser [46]. The desired shape, geometry, and dimensions of MNDs are created using some of the computer-aided design (CAD) software. The laser beam follows the predetermined shape of the needle, and then, MNDs are cleaned in hot water and bent at 90 degrees, vertically from the plane of the base. In order to deburr, reduce the thickness of MNDs, and sharpen the tips, MNDs are subsequently electropolished, washed, and dried with compressed air. This manufacturing method can be employed to produce a single row of MNDs of different geometries, as well as 2D rows of metallic MNDs [53,59,120–122].

Between the most recent works stand out the methodology proposed by Albarahmieh et al. [123], which reported on the fabrication of MND patches using a CO_2 laser on polymethylmethacrylate (PMMA) sheets. Subsequently, a selected mixture was poured into PMMA molds, and then, dissolving MNDs, containing methylhydroxy-4-benzoate and terbinafine hydrochloride, were successfully obtained [46].

6.1.2 Laser ablation

In the same way for laser cutting, the laser ablation is also used for fabricating MNDs based on metal or polymer [124]. Laser cutting involves cutting a metal or polymer plate into a 2D shape, whereas laser ablation engraves the plate into a 3D shape. Basically, when the substrate is irradiated with a laser beam (e.g., CO_2 laser beam), it absorbs the laser energy and heats, resulting in its evaporation or sublimation. Through this process, an inverse mold can be produced by generating an MND pattern [117]. It is worth to consider that, due to the high-intensity laser pulses, the formation of plasma of ions and electrons is not suitable for the fabrication of structured materials.

In 2020, Evens et al. [125] introduced a novel method to produce solid polymer MNDs using laser-ablated steel molds. This mold was also

employed in the injection molding process to produce polymeric MNDs. In this way, the height of MNDs can be varied, and a sharp tip radius can be obtained using this low-cost production method.

6.2 Photolithography

Photolithography is the methodology employed to create solid or hollow MND. This method is used to manufacture silicon MND or dissolving/hydrogel MND by making an inverse mold based on the MND structure. When fabricating silicon MND using photolithography, a sacrificial layer is deposited in the form of a thin film on cleanly treated silicon [117]. Subsequently, a photoresist, made of a photosensitive polymer, is coated on the silicon via spin coating. If the photomask with desirable pattern is aligned on the substrate and exposed to strong UV radiation, the desired pattern is generated in the exposed or not exposed part. The pattern is generated in the photoresist through the development process. Subsequently, the exposed substrate without the photoresist is etched through the etching step, which will be reviewed in the next section. So, a desirable pattern is transferred from the photomask to the photoresist to the silicon [126,127].

6.2.1 Etching

As mentioned earlier, when an MND is fabricated using general photolithography, etching is an important process for determining the tapered shape of the MND tip [117]. Before the etching process, the size of the MND base and the gap among the MNDs are determined. Then, the length and shape of the MNDs are defined through the etching process [128]. The etching process is classified as dry etching and wet etching; in this way, it results in isotropic or anisotropic etching, depending on the utilized method.

6.2.2 Dry etching

Dry etching is the technique used to create solid or hollow MND and can be classified into physical methods and chemical methods. Physical methods include ion milling and sputtering [44,129]. In dry etching, an inert gas as argon is ionized by high energy and unidirectional electrodes, so that the ions strike the silicon substrate at a high speed in a single direction, thus anisotropic etching is performed. In the manufacturing process, the area protected by the oxide film (sacrificial layer), or photoresist is hardly etched, while the exposed area on the silicon is etched [117]. Chemical methods include high pressure plasma etching, in which a chemically reactive plasma gas is generated using strong energy. The plasma reacts with the surface of

the substrate, and it is converted into a volatile material, which is blown away, thereby resulting in isotropic etching of the substrate. Reactive ion etching combines physical and chemical methods; both plasma and sputter etching can be used to control isotropic and anisotropic etching [87]. Through the optimization of this process, a precise MND sharp tip can be manufactured [43].

6.2.3 Wet etching

Wet etching is another option for the fabrication of metal or silicon MNDs [128]. In this process, a pattern is produced on the substrate using a chemical etchant. In the case of a silicon wafer, a potassium hydroxide aqueous solution is used. A sharp tip shape can be produced by applying different rates of etching, depending on the direction of the silicon crystals [43,130]. Wet etching is primarily isotropic etching via a chemical reaction; the etching rate is significantly faster than that in dry etching [117]. Although the required cost for the entire process is low, the poor accuracy of this method is a disadvantage for the fabrication of fine patterns [129].

6.3 3D printing

The 3D printing is a new technology that rapidly prototypes a design at low cost and high throughput [130]. Recently, 3D printing technology has been expanded to include the production of microstructures such as MNDs [131,132]. Nowadays, the existing manufacturing technology is limited to the production of a simple structured MND, while the emerging 3D printing technology can produce a more sophisticated and complex-shaped MND structure [133]. MNDs are manufactured using high precision stereolithography, digital light processing method, or fused deposition modeling [117].

6.4 Microstereolithography (μSL)

The manufacturing of 3D objects using the μSL method is based on the photopolymerization of a liquid resin using a light source such as UV radiation and the process of controlling the space to manufacture the 3D object. The building stage and laser beam or digital light projector are precisely controlled by a computer so that the resin surface is illuminated by light. A layer-by-layer is created on the surface of the building platform, forming the structure [134,135]. For example, an MND based on poly(propylene fumarate) was prepared using μSL technology for the treatment of skin cancer [117]. To improve mechanical strength, a biodegradable polymer,

poly(propylene fumarate), was mixed with diethyl fumarate. This MND system enabled controlled release of dacarbazine, an anticancer drug, for 5 weeks through modification of the drug dose and molecular weight of the polymer monomer [136].

6.5 Continuous liquid interface production

Continuous liquid interface production (CLIP) is a methodology used to fabricate objects through photopolymerization of a photoreactive resin using the reflected light from a general digital light processing (DLP) chip [137]. The basic principle of the CLIP is the same as that for the DLP method; however, CLIP addresses the problem of peeling of the cured resin layer. Since the separation and rearrangement steps, which are rate limiting in the conventional process, have been eliminated, the MND could be produced in approximately 2 to 10 min (i.e., reducing the output time by approximately 25 to 100 times compared to that for the conventional method) [138]. A recent example by Johnson et al. [137] report CLIP-based production of MND arrays using biocompatible polymers (i.e., trimethylolpropane triacrylate, polyethylene glycol dimethacrylate, polycaprolactone trimethacrylate, and polyacrylic acid) [137].

6.6 Two-photon polymerization

Two-photon polymerization (TPP) is a modern additive manufacturing method with a resolution of approximately 100 nm [139]. TPP initiates polymerization of the resin through multiphoton absorption, which occurs through excitation of the photoinitiator [117]. TPP employs a near-infrared wavelength laser, such as a titanium-sapphire laser, instead of UV light. In the TPP method, unlike in the conventional high precision stereolithography method, the curing reaction does not occur in the illumination path of the entire laser beam but only at the focal point [140]. Therefore, it is possible to manufacture elaborate and complex 3D structures [141,142].

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CHAPTER 4

Stimuli-responsive microneedles for drug delivery

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1 Introduction

The conventional methods employed for drug delivery, namely, oral and parenteral routes, are often limited by the physicochemical characteristics of the drug. For instance, oral administration is linked with first-pass metabolism, which significantly diminishes the bioavailability of the administered therapeutics. Injections are invasive and necessitate administration by skilled personnel, which can be excruciating and potentially result in needle phobia in patients, particularly in children [1,2]. In contrast, MNs have the ability to permeate the outermost stratum of the skin (stratum corneum) to locally administer bioactive agents into the epidermis and/or superficial dermis. Microneedles afford a pain-free administration approach that can be self-administered by patients [3,4].

In recent years, the field of microneedle technology has witnessed significant advancements. There has been a surge in the publication of novel microneedle-based materials, particularly polymers, from a diverse array of microneedles [5]. However, the drug-release behaviors of MNs typically lack adequate control and are primarily reliant on the inherent parameters of MNs. Furthermore, numerous chronic ailments necessitate long-term and repeated administrations, thereby creating an urgent demand for controllable MNs to achieve on-demand drug release. To enhance the efficacy of disease treatment and diagnosis through synergizing the benefits of MNs, the concept of intelligent stimulus-responsive MNs has been introduced. These MNs have demonstrated a prominent role in sustainably controlled drug delivery for disease therapy in recent times. Compared with conventional MNs, these intelligent stimuli-responsive MNs can be employed to deliver drugs in a more controllable manner for achieving on-demand and sustained administration [6,7]. This chapter highlights various examples of intelligent stimuli-responsive MNs for drug delivery. In Fig. 1, we have summarized the contents of this chapter.

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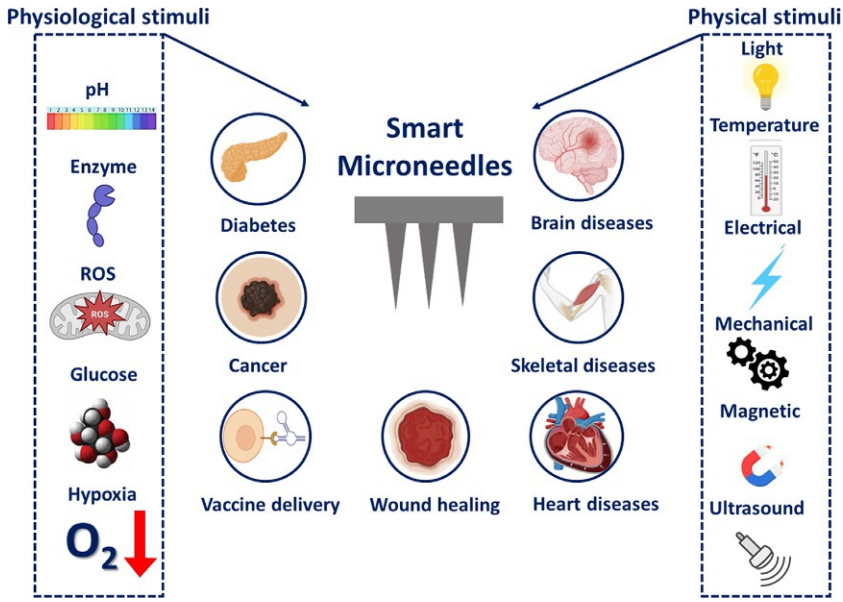

Fig. 1 Different type of stimuli and application of smart microneedle in drug delivery.

2 Stimulation of biomaterials

"Intelligent" materials, also known as responsive materials, exhibit the ability to modify certain variables in response to changes in their environment. These alterations can impact the mechanical, electrical, or visual characteristics of the material, as well as its composition, structure, or function [8]. In particular, smart bioresponsive materials are highly beneficial for the development of precise medications due to their sensitivity to biological signals and their capacity to interact with or be stimulated by them [9]. The concept of employing stimuli-responsive drug delivery was initially introduced in the late 1970s, when thermosensitive liposomes were utilized to achieve localized drug release via hyperthermia. Since this time, extensive research has been conducted on stimuli-responsive materials for drug delivery, particularly in the past decade [10]. Various types of stimuli, both endogenous and exogenous, have been examined for the purpose of producing advanced and bioinspired nanosystems with functional properties that are capable of responding to microenvironmental conditions. These stimuli may originate from natural sources within the human body or external artificial sources.

Stimuli may be categorized into intracellular and extracellular, physical, chemical/biochemical, and biological/physiological categories. From both

external sources and internal conditions, shear stress and temperature fluctuations are stimuli that may emerge [11–13]. To achieve the desired applications, it is crucial to choose the appropriate stimuli-responsive mechanism(s) that exhibit highly selective and sensitive target-specific responses. Physical changes, chemical changes, or a combination of both, which are generally based on basic building blocks, can form the stimuli-triggered responses. Typical bioresponsive actions include degradation, detachment, uncap, charge reversal, dissociation, separation, and cross-linking [9].

2.1 Physical stimuli

2.1.1 Light

Light-responsive materials are composed of a photosensitive molecule, such as azobenzene, spiropyran, or dithienylethene, or employ photocleavable crosslinkers like o-nitrobenzyl esters, to detect the presence of light [14,15]. Upon illumination, the molecular conformation, configuration, or constitution of the photo activated moiety is altered, resulting in changes in the chemical character of the compound, such as isomerization form, polarity, hydrophobicity, or crosslinking density. These changes are reflected as a macroscopic transformation in the material. The use of photostimulation is particularly attractive for biological applications as it allows for contact-free, precise, and remote control of the region of actuation without causing any invasive or harmful effects on cells [14,16]. Currently, a range of illuminating mechanisms are under investigation and implementation, encompassing ultraviolet (UV), visible, near-infrared (NIR), and diverse laser types with varying wavelengths. In the realm of biomaterials that are light responsive, UV light has been a customary means of instigating drug release. This is because of the heightened sensitivity of such materials to shorter wavelengths of light. Nevertheless, NIR-responsive agents are garnering increased popularity in comparison to UV-responsive agents, as the latter's capacity to penetrate tissues is limited [17].

2.1.2 Temperature

Thermoresponsive materials are characterized by their ability to alter their properties in response to changes in temperature within their environment. Once the temperature reaches a specific threshold, commonly referred to as the critical temperature, the conformation of the polymers undergoes a transformation, ultimately resulting in a shift in solubility. It is noteworthy to mention that thermoresponsive polymers can possess either an upper

critical solution temperature (UCST) or a lower critical solution temperature (LCST). It is important to note that below this threshold, polymers possessing a UCST are rendered insoluble, whereas those with an LCST exhibit solubility [18]. Polymers have been utilized for the construction of bioresponsive materials aimed at achieving sustained drug release, primarily due to their temperature-triggered characteristics. By adjusting the ratios of hydrophobic monomers or modifying the types of end groups of temperature-responsive polymers, it is feasible to easily regulate the lower critical solution temperature (LCST) of polymers [18]. Polymers that possess an LCST ranging from room temperature (25°C) to body temperature (37°C) are deemed to be optimal candidates for temperature-responsive materials [19].

2.1.3 Electrical

Electroresponsive materials, composed of conductive polymers or embedded with conductive particles or ionic liquids, are capable of reacting to external electrical fields [20]. In biomechanics, these materials serve a valuable purpose due to their ability to convert electrical energy into mechanical energy. As a result, electroresponsive polymers can cause materials to undergo expansion, contraction, or bending when exposed to an electric field [21,22].

2.1.4 Mechanical

Mechanical forces, including compressive, tensile, and shear forces, are present extensively in the human body and can be externally imposed [23]. The mechanical force-sensitive delivery systems have received significant attention due to their ability to provide a practical means for controlled drug release. Such systems allow patients to self-administer therapeutics through mechanical force-mediated triggers that respond to mechanical strains caused by basic body movements, including muscle tension, organ function, tendon activity, and bone articulation. These modalities promote spatiotemporal drug release [24]. The application of mechanical strain-based stimuli during routine bodily movements presents a straightforward and easily accessible method for achieving spatiotemporal drug release. The introduction of strain variations can be accomplished by means of muscle, organ, tendon, and bone joint tension, cartilage and bone compression, or blood vessel shear forces. In contrast to magnetic field-responsive MNs, light, and electric current, the release rate of drugs from mechanical force-responsive MNs can be accurately regulated through simple self-administered actions such as stretching, bending, pressing, or insertion. This level of control is possible regardless of the drug's structure, whether it is anionic/cationic/large

molecules/thermal-sensitive drugs. Consequently, this MN system has the potential to be a highly effective, uncomplicated, and cost-effective tool for on-demand drug delivery that can be self-administered [25].

2.1.5 Magnetic

Magnetically responsive materials possess the ability to respond to a magnetic field. These materials consist of ferromagnetic configurations, such as metallic particles or nanowires, that are typically comprised of elements such as Fe, Co, and Ni, mixed with polymers like PDMS or collagen to create a variety of compounds [25]. When a magnetic field is applied, the embedded magnetic structures within the material reorganize, leading to changes in the material's topography. Applying a magnetic field to a magnetoresponsive material can also alter its viscosity, stiffness, and yield stress within a short time frame of 3 min due to the formation of a chain structure by the magnetic particles in the material, which causes the material to actuate. Once the magnet is removed, the material returns to its original state, enabling fast, tunable, and reversible modulation of the material [26–28].

2.1.6 Ultrasound

Ultrasounds can be produced by applying an alternating current, which generates high-frequency waves that can have both thermal and nonthermal effects. The nonthermal effects include the formation of small gas bubbles, which can cause an increase in pressure and temperature. Alterations in mechanical properties can occur due to changes in the material caused by the bubbles' formation and subsequent collapse. Ultrasounds have several potential applications, including the disruption of small vesicles such as micelles or nanocarriers to release their cargo, such as anticancer drugs, or the disruption of hydrogel bonds or facilitation of a phase change in the gel material [29]. Furthermore, the introduction of ultrasounds can be conveniently achieved through the utilization of an ultrasound transducer [30].

2.2 Physiological stimuli

Physical stimuli can be useful in materials due to their ability to provide local and remote control, but they need continuous illumination to maintain a continuous response [31]. Besides physical stimuli, chemical signals can also be employed in combination to provoke a response from a material. Bioresponsive materials that respond to biological signals can be designed to target physiological parameters that are important indicators of various diseases, including autoimmune disorders, cancer, infections, degenerative diseases,

and cardiovascular diseases. As a result, these physiological parameters present themselves as compelling objectives in the process of developing bioresponsive materials. pH, redox, enzyme, glucose, and hypoxia (insufficient levels of oxygen within the tissues) are typical physiological stimuli [9].

2.2.1 pH

The variation in pH levels has been utilized to regulate the administration of drugs, in particular organs or intracellular spaces. The utilization of this technique has also been employed to initiate the discharge of medicaments in reaction to slight variations in environmental factors that are linked with pathological states including cancer and inflammation. Two primary techniques are employed for this purpose: the utilization of polymers, specifically polyacids or polybases, containing ionizable groups that experience alterations in conformation and/or solubility as a result of modifications in pH levels. The development of polymer-based systems that exhibit bonds sensitive to acidic conditions, capable of cleaving and releasing molecules attached to the polymer backbone, altering the polymer's charge or exposing targeting ligands, is a significant area of research [32].

Materials that are pH sensitive can undergo chemical or physical changes, including shrinking, dissociation, swelling, degradation, or membrane fusion and disruption (Fig. 2) [33]. Changes in pH levels can cause chemical and physical alterations that result in the swelling of the polymeric system, leading to the release of the enclosed drug [34].

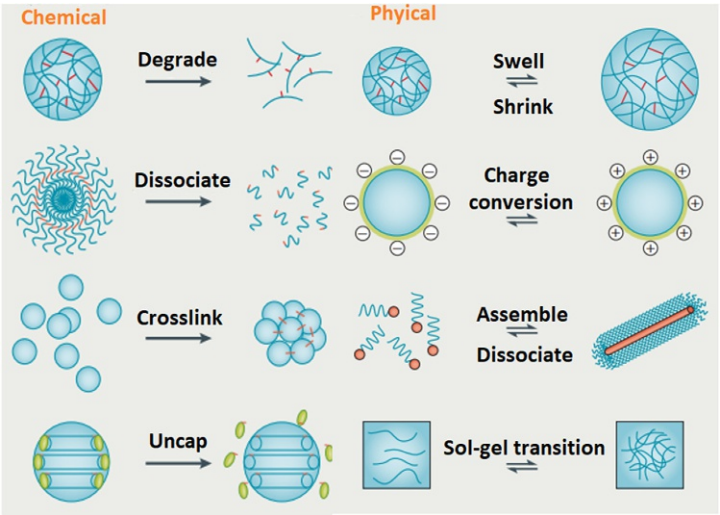

Fig. 2 Typical bioresponsive actions.

2.2.2 Enzyme

The uniqueness of enzyme sensitivity lies in the high selectivity of enzymes in their reactivity, their ability to function under mild conditions that are present in living organisms, and their critical role in many biological processes [35]. Enzyme-responsive materials are generally composed of a substrate that exhibits sensitivity toward enzymes and an additional component that controls or regulates the ensuing interactions leading to macroscopic transitions. The enzymatic catalytic activity upon the substrate can result in modifications of supramolecular architectures, the expansion or contraction of gels, or variations in surface properties [36].

2.2.3 Glucose

Polymeric systems responsive to glucose are often developed utilizing glucose oxidase (GOx) to enzymatically oxidize glucose, along with the interaction between glucose and concanavalin A (ConA), as well as the reversible formation of covalent bonds between glucose and boronic acids [37]. In these systems, insulin molecules are commonly attached to a support or carrier through specific interactions, which may be hindered by glucose. This mechanism typically involves the incorporation of functional groups within insulin molecules [38]. Most investigations on polymers responsive to glucose are based on the reaction between glucose and oxygen, facilitated by glucose oxidase (GOx). The sensitivity of these polymers to glucose usually arises from the response of the polymer to by-products generated during glucose oxidation, rather than a direct interaction between glucose and the responsive polymer. The catalytic function of glucose oxidase (GOx) on glucose is highly specific, resulting in the production of secondary metabolites such as gluconic acid and hydrogen peroxide (H_2O_2). Incorporating a polymer that reacts to either of these by-products may yield a system responsive to glucose [39]. An alternative class of glucose-responsive systems employs the mechanism of competitive binding of glucose with glycopolymer–lectin complexes. Lectins, a class of proteins with the unique ability to specifically bind carbohydrates, tend to induce cross-linking and/or aggregation of glycopolymers due to their multivalent nature. However, introducing a saccharide with competitive binding properties can effectively disrupt this aggregation process [40].

An alternative mechanism for glucose response involves the use of polymers that are made solely from synthetic components, specifically boronic acid-based glucose-responsive polymers, such as PBA. These types of polymers have been employed for insulin delivery. The underlying concept of glucose-responsive drug delivery facilitated by PBA is predicated upon

the reversible interaction between PBA and cis-diols. Phenyl boronic acid, a synthetic Lewis acid, exhibits reversible binding affinity to hydroxyl groups in glucose, leading to the formation of cyclic esters mediated by its phenyl borate moieties. The pH-responsive property of select materials is exploited in the development of self-regulated drug delivery systems, which operate by monitoring blood glucose levels. Typically, these systems use PBA (phenylboronic acid) moieties, which are negatively charged and relatively hydrophilic when the pH of PBA (8.2–8.6) exceeds its pKa, the PBA molecules bear a negative charge and have a relatively high affinity for water. Conversely, if the pH of the solution drops below PBA's pKa, the PBA molecules lose their charge and become hydrophobic. The alteration in hydrophilicity pertaining to materials functionalized with PBA instigates the process of either swelling or shrinking of platforms based on PBA, which subsequently exerts an impact on the profile of drug release [41,42].

2.2.4 ROS

In various pathological states, such as cancer, stroke, arteriosclerosis, and tissue damage, an imbalance in the oxidation-reduction process often leads to elevated levels of reactive oxygen species (ROS). ROS can serve as useful markers for identifying the location of targeted treatment [43]. ROS-sensitive polymers can be classified into two primary categories: those that undergo a noncleavable hydrophobic-hydrophilic transition induced by ROS, and those that undergo structural cleavage induced by ROS. In the first category, ROS catalyze the oxidation process of chalcogen elements such as S, Se, and Te, altering their valence. Polarized groups within the material provide hydrogen for water binding, and oxygen can adhere to chalcogen constituents. The alteration in the substance's hydrophilicity/hydrophobicity is a direct consequence of this process. In the second category, polymers are subjected to ROS and undergo chemical bond cleavage. Commonly employed ROS-sensitive polymers include thioketals, phenylboronic acids/esters (PBAs/PBEs), vinyl di-thioethers, and proline oligomers [44]. Materials that are responsive to redox changes can react to the presence of oxidants or reductants in the surrounding media or introduced through an external voltage. To facilitate reactions to alterations in the redox state, materials must contain disulfides, organometallics, viologens, or tetrathiafulvalenes [45]. Redox-responsive materials are particularly useful as drug delivery systems for anticancer therapies, as tumor cells tend to generate a reducing microenvironment due to the augmentation of glutathione synthesis [46]. Alterations in the redox condition of the microenvironment may

impact the robustness of the polymer cross-links within drug carriers, thus modifying the configuration and penetrability of the conveyance mechanism and ultimately culminating in the release of the drug cargo [47–49].

2.2.5 Hypoxia

Hypoxia, characterized by a reduction in the oxygen supply to support metabolism, can occur locally or systemically within the body. Hypoxia has emerged as a crucial parameter in assessing cancer advancement and is implicated in many other pathological states, such as antiinflammatory responses, wound healing, neurodegeneration, and mountain sickness. In hypoxic areas, cells attempt to adapt to unfavorable conditions by activating genes associated with glucose metabolism and transport, as well as angiogenic and growth factor production. These genes can enhance oxygen transportation by triggering angiogenesis and elevating the production of red blood cells. Hypoxia-inducible factor-1 (HIF-1) plays a crucial role in regulating hypoxia-inducible transcription. Its functionality is scarcely distinguishable in ordinary cells but activated in hypoxic conditions. Based on this fundamental principle, the effectiveness of a therapeutic gene containing HREs governed by the transcriptional regulation of HIF-1 has been extensively researched and employed in hypoxia-responsive gene therapies for a diverse range of conditions [50].

2.3 Multiple stimuli

Multiresponsive materials possess the capability to react to a wide range of external stimuli, either independently or collectively. This particular characteristic enables the induction of a plethora of material alterations with profound accuracy and specificity. The utilization of multiresponsive materials bears a captivating potential to introduce multidimensional cues to cells, thus facilitating the analysis of their responses. By amalgamating diverse forms of polymers, thermomagnetic, thermolight, or thermo pH-responsive materials have been formulated, which demonstrate promising prospects in tissue engineering strategies and sensors [51].

3 Stimuli-responsive drug delivery MNs

MN patches, which comprise small needles, have emerged as a potentially valuable tool for creating perforations in the stratum corneum and facilitating the translocation of biomolecules into the dermis in a minimally invasive manner. Among these, stimuli-responsive MN patches are an emerging class

of drug delivery systems that have the capability to release their contents in response to both internal and external stimuli. This feature enables the controlled and targeted delivery of drugs, making them a promising modality for drug delivery purposes [52]. MNs have been developed, which are typically composed of polymeric matrices. These materials are designed to respond to changes in the surrounding environment and can thus facilitate and regulate the release of drugs or other micro cargos. Stimuli-responsive materials encompass a broad range of compounds that are capable of responding to various environmental cues. As a result, they offer precise control over the release of drugs or other cargos and can be adjusted based on the physiological environment or recommended dosage, thereby reducing the risk of side effects due to excessive or inadequate release [53].

3.1 Single stimuli-responsive MN

Stimuli-responsive microneedle patches are a promising technology that can be used for on-demand drug delivery to improve treatment efficacy and reduce systemic side effects. By responding to various physiological or external stimuli, these systems can be triggered to release drugs in a controlled manner. Microneedles that are triggered by physiological signals have the added benefit of self-regulated/closed-loop drug delivery, which enables them to respond to changes in physiological signals and optimize therapeutic efficacy. This approach has the potential to improve patient compliance and treatment outcomes. However, microneedles that are triggered by external physical stimuli often require an external source, which can be costly and not easily accessible for patients. Portable units for administering these external stimuli may not be readily available, limiting their wider use [33]. Unlike their conventional MN counterparts, these innovative MNs are constructed with polymeric materials that exhibit swelling, disintegration, or dissolution solely in reaction to a specific triggering system. This feature enables the controlled and targeted release of drugs, which can improve treatment efficacy and reduce systemic side effects [52].

In the year 2005, the notion of MNs that are responsive to stimuli was introduced by Zahn et al. These MNs can effectively deliver insulin in reaction to a surge in blood glucose levels, thus generating an artificial pancreas that operates in a closed-loop manner [54]. A study conducted by Chen et al. a decade later illustrated the first instance of stimuli-responsive MNs in a proof-of-concept demonstration. The development of their system involved the use of silica-coated lanthanum hexaboride nanoparticles that

were embedded in polycaprolactone-based polymeric MNs. These MNs facilitated the delivery of doxorubicin in response to externally applied near-infrared light [55].

3.2 Hybrid stimuli-responsive MN

In recent years, it has been demonstrated by both exogenous and endogenous stimuli-responsive MNs that on-demand drug delivery is of utmost significance in the effective treatment of diverse disease conditions. Although single stimuli-responsive MNs can achieve on-demand drug delivery, certain disease conditions necessitate precise on-demand drug delivery. Consequently, many researchers have become interested in enhancing the effectiveness of drug treatment by combining two responsive drug delivery features into a single MN system. Single stimuli-responsive MNs are developed to address particular medical conditions. In contrast, dual stimuli-responsive MNs have the capability to treat several different diseases using only one MN patch. This technology can be particularly beneficial for patients with superficial tumors who may experience varying levels of discomfort as a cancer symptom or a side effect of cancer treatment. When faced with such situations, dual stimuli-responsive MNs can be employed to treat surface tumors through one stimulus while simultaneously managing pain using a separate stimulus. This approach represents a promising avenue for improving treatment outcomes and reducing patient discomfort [56].

4 Applications of stimuli-responsive MN

4.1 Diabetes

Diabetes is a pathological condition that is distinguished by a deficiency in the capacity to regulate glucose levels within the body. The conventional treatment method for this condition typically entails the administration of frequent subcutaneous injections of insulin to maintain blood glucose levels within the standard range of 100–200 mg/dL [57]. Nevertheless, it is noteworthy that this therapeutic approach is frequently associated with excruciating pain and inadequate glucose control, which further elevates the risk of complications such as limb amputation, blindness, and kidney failure [58]. Conventional MNs possess the capacity to administer insulin devoid of inflicting pain. Regrettably, the "open-loop" technique of insulin delivery utilizing MNs can lead to hypoglycemia or hyperglycemia due to the fluctuations in blood glucose levels experienced by diabetic individuals. Hence,

a self-responsive insulin delivery system that exhibits high sensitivity to blood glucose level changes is indispensable for attaining optimal glycemic control among diabetic patients [59]. In the subsequent discourse, we shall expound upon the various classifications of intelligent microneedles that have been devised for the therapeutic management of diabetes.

4.1.1 Glucose-responsive MNs

Glucose-responsive MNs have shown great promise in the delivery of anti-diabetic drugs, specifically insulin and metformin, for the treatment of diabetes. These microneedles are classified as "smart" due to their ability to react to changes in blood glucose levels. High levels of blood glucose initiate a reaction catalyzed by glucose oxidase (GOx), which rapidly consumes dissolved oxygen, causing the dissociation of glucose-responsive microneedles and the subsequent release of insulin. This mechanism allows for instant, glucose-responsive, and safe closed-loop insulin delivery [57]. Currently, there are several types of glucose-responsive MN systems operating on different principles that are available for use, including the following:

(i) The implementation of electronic glucose-monitoring sensor-based MNs is a promising approach to detect and respond to high blood glucose levels by delivering insulin in a timely manner [60,61].

(ii) Glucose oxidase (GOx) enzyme-based MNs have been developed to respond to environmental changes catalyzed by GOx, such as acidic pH or hypoxia, and trigger the release of insulin in response to glucose levels [62].

(iii) Phenylboronic acid (PBA) chemical-based MNs exhibit reversible swelling in response to hyperglycemia, enabling a diffusion control mechanism for the delivery of encapsulated insulin [63].

Yu et al. have successfully developed a novel glucose-responsive MN patch that contains glucose-responsive vesicles (GRVs) encapsulated with insulin and glucose oxidase (GOx) enzyme (Fig. 3A and B). These GRVs are self-assembled from hypoxia-sensitive hyaluronic acid (HS-HA) conjugated with 2-nitroimidazole (NI), which is a hydrophobic component that can be transformed into hydrophilic 2-aminoimidazoles through bioreduction under hypoxic conditions. In the hyperglycemic state, enzymatic oxidation of glucose leads to a local hypoxic microenvironment, which promotes the reduction of HS-HA, thereby triggering the dissociation of vesicles and subsequent release of insulin in a rapid manner. Animal studies have demonstrated that diabetic mice treated with GRV-loaded MNs maintained normoglycemic levels for up to 4 h before gradually increasing [64].

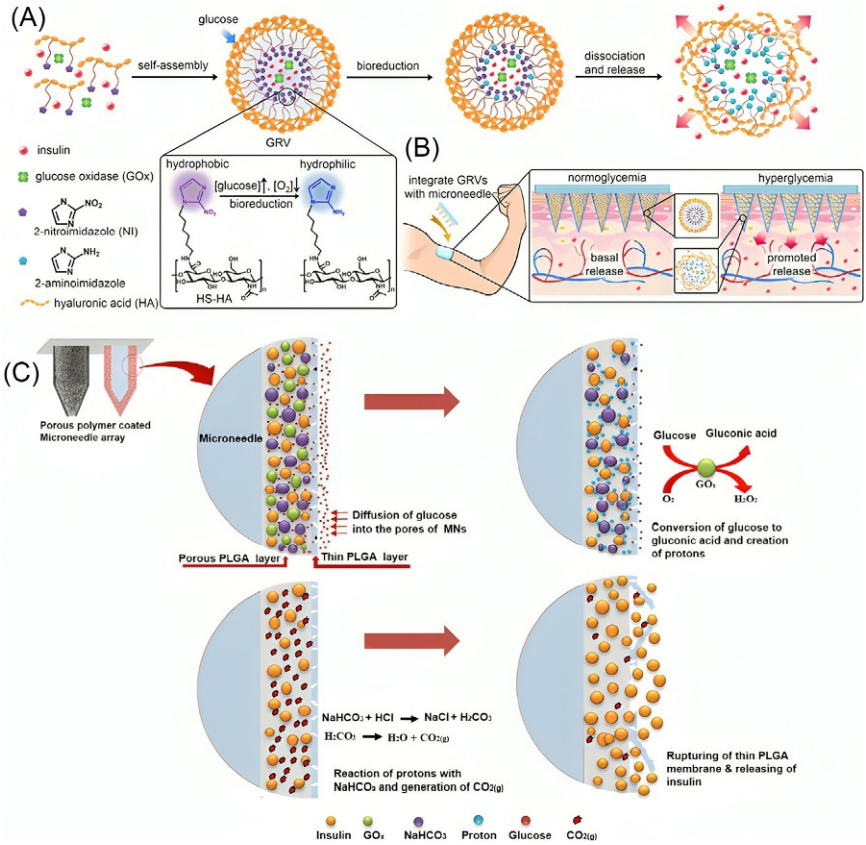

Fig. 3 (A) Schematic of the glucose-responsive insulin delivery system using hypoxia-sensitive vesicle-loading MN-array patches. Formation and mechanism of GRVs composed of HS-HA. (B) Schematic of the GRV-containing MN-array patch (smart insulin patch) for in vivo insulin delivery triggered by a hyperglycemic state to release more insulin [64]. (C) Schematic demonstration of the glucose-responsive insulin delivery system using porous-coated MNs [65].

Yu et al. have developed an innovative MN design for the purpose of insulin delivery in response to elevated blood glucose levels. The MNs are equipped with "glucose 1" (GSA) nanovesicles at their tips, which contain GOx, glucoamylase (GA), and α-amylase (AM). The GSA nano vesicles work in concert to amplify the local glucose concentration, inducing insulin secretion and delivery through the MNs when exposed to high blood glucose levels. With a loading capacity of approximately 7.4 wt% and a loading efficiency of around 16.1 wt%, the diabetic mice treated with the MN systems experienced a normoglycemic period of roughly 6 h, which can be prolonged

by using an additional MN patch without causing hypoglycemia [66]. Chen and colleagues have developed an innovative patch of glucose-responsive MN that incorporates two types of biomineralized protein particles, namely, mineralized Ex4 (m-Ex4) and mineralized GOx (m-GOx) particles. In the case of hyperglycemia, the m-GOx enzyme would be activated, thereby stimulating the release of Ex4 from the pH-sensitive m-Ex4 particles contained within the MNs. These dual-mineralized particle-containing MNs exhibited an exceptional hypoglycemic effect. They were capable of sustaining a normoglycemic state for nearly 12 h, and even after 72 h of treatment, the blood glucose levels of the mice remained below the original level [67].

Ullah and colleagues have recently developed an MN patch that responds to glucose levels to deliver insulin via the transdermal route when hyperglycemic conditions are present. The MNs are composed of stainless steel with a poly (lactic-*co*-glycolic acid) (PLGA)-based polymer coating, which contains numerous pores that can hold GOx, sodium bicarbonate ($NaHCO_3$), and insulin. An additional layer of PLGA is applied to cover the glucose-responsive, pH-sensitive, and insulin-embedded pores to prevent insulin release during normoglycemic states (Fig. 3C). When blood glucose levels increase, glucose passively diffuses into the porous MNs and is enzymatically converted into gluconic acid. Simultaneously, $NaHCO_3$ decomposes to generate carbon dioxide (CO_2) gas. The generation of CO_2 gas has the potential to induce pressure within the pores of the system, which can lead to the distortion of the thin PLGA layer and an eventual release of the loaded insulin; in diabetic rodents, hyperglycemia was rapidly counteracted within the initial 2 h of treatment, and normoglycemia (100 ± 20 mg/dL) was maintained for a duration of up to 10 h [65]. Hsu and colleagues have developed a novel MN system that is capable of both diagnosing and treating diabetes. The MN set comprises two components: a glucose-biosensing MN patch and an insulin-delivery microneedle patch. The glucose-biosensing microneedle patch (GBMP) is composed of a combination of GOx-conjugated MnO_2/graphene oxide enzymes (GOx-MnO_2@GO) and methacrylate gelatin that swells upon contact with interstitial fluid from the epidermal layer. Notably, the hyperglycemic response of the GBMP allows for color development that can be detected through a smartphone, thus enabling quantification of blood glucose levels. What distinguishes this system from others is that it does not require a high concentration of GOx to trigger insulin release via pH alteration during hyperglycemia [68]. To avert hypoglycemia that can result from excessive insulin administration, a novel approach employing glucose-responsive

MN has been developed. The MNs are engineered to release native glucagon, which is dispersed in an MN matrix consisting of photocrosslinked methacrylate hyaluronic acid. Encapsulation of glucagon is necessary due to its susceptibility to chemical degradation and physical denaturation under physiological conditions. During hyperglycemia or euglycemia, the 4-acrylamide-3-fluorophenyl boronic acid (AFBA) component induces microgel swelling. Conversely, during hypoglycemia, the concentration of glucose falls below that of AFBA. Biscomplexation of AFBA with glucose then triggers secondary crosslinking between the polymer segments, leading to the shrinkage of the microgel and the release of the loaded glucagon. In transdermal diabetic rat skin application, the MNs proved effective in preventing hypoglycemia [69].

Gu et al. have developed a hybrid glucose-responsive patch that functions as an artificial pancreas. This synthetic patch is capable of delivering insulin and glucagon in a glucose-dependent manner, utilizing dual modules that imitate the regulatory function of pancreatic islet cells in modulating blood glucose levels. The modules were synthesized through photopolymerization of the same monomers with varying ratios, resulting in "dually responsive" modules that can release payloads in response to either hyper- or hypoglycemic conditions. The dual glucose responsiveness of insulin and glucagon delivery arises from the synergistic net charge changes of the polymeric matrix at different glucose concentrations, the expansion or contraction of the matrix, and the disparity in isoelectric points of the insulin and glucagon molecules at physiological pH [70]. In order to confront the obstacles associated with tissue damage resulting from local hypoxia, Luo and colleagues have devised a glucose-responsive MN patch that encompasses ultra pH-sensitive nanoparticles (NPs) containing both GOx and catalase (CAT) enzymes to dispense insulin accordingly. The initial step involved the encapsulation of insulin into pH-sensitive NPs (SNP (I)) consisting of poly (ethylene glycol)-b-PHMEMA. Subsequently, GOx and CAT enzymes were incorporated into pH-insensitive NPs (iSNP (G+C)) composed of poly (ethylene glycol)-b-poly (2-cyclohexyl ethyl methacrylate). Finally, the present study reports the loading of both pH-sensitive and pH-insensitive nanoparticles (NPs) into polyvinylpyrrolidone (PVP)-based microneedles (MNs). Upon encountering high blood glucose levels, the pH-insensitive NPs containing glucose oxidase (GOx) would catalyze the conversion of glucose to gluconic acid, inducing a localized acidic microenvironment. This would subsequently lead to the protonation of tertiary amino groups in the poly (2-(dimethylamino)ethyl methacrylate)

(pHMEMA) structure, thereby triggering the breakdown of pH-sensitive NPs, and consequently releasing insulin. Moreover, the catalase (CAT) enzyme would effectively consume excess H_2O_2, thereby minimizing potential tissue damage. Impressively, the developed MN patch could effectively restore blood glucose levels to normal in an STZ-induced type 1 diabetic mice model for as long as 10 h. However, the use of iSNP (I) and SNP (G + C) loaded MNs was found to significantly reduce high blood glucose levels within 2 h, albeit with a subsequent rapid increase in blood glucose levels thereafter [71].

We have successfully created a novel MN patch that is capable of reacting promptly to hyperglycemia/normoglycemia in order to deliver insulin without any risk of dissociation or degradation of the MN. The patch is equipped with macroporous MNs (MMNs) that are primarily composed of alumina and serve as an efficient core material to accommodate a large quantity of insulin. A shell-like structure with numerous pores is formed by coating a thin layer of mesoporous silica (MS) onto the MMNs. Following this, a layer of chitosan (CS) hydrogel containing GOx/CAT enzymes is applied to the MS layer to regulate insulin delivery under hyperglycemic/normoglycemic conditions. The CS in this system functions as a gatekeeper, and the "swelling/deswelling" of CS can be interpreted as "cap doffing/donning" during hyperglycemia/normoglycemia. During periods of elevated blood glucose levels, the enzymatic conversion of glucose into gluconic acid may result in the protonation of chitosan (CS) chains, triggering the "cap doffing" mechanism that facilitates the release of insulin from microneedles. Additionally, the enzyme CAT functions to convert produced H_2O_2 to both water and oxygen, thereby preventing local hypoxia conditions. Conversely, during normoglycemic conditions, neutralization of CS chains prompts "cap donning," which obstructs insulin release. In vitro investigations have demonstrated that the CS hydrogel experiences repeated cycles of swelling and deswelling in hyperglycemic and normoglycemic states with 40-min intervals. The intelligent MN patch that has been developed induces normoglycemia in type 1 diabetic mice within an hour and sustains it for an additional 5 h [72]. Seong and colleagues have developed an innovative technique for the transdermal delivery of insulin. This technique involves the use of bullet-shaped double-layered MN arrays with water-swellable tips. The design of these MNs enables them to mechanically interlock with soft tissues through selective distal swelling post skin insertion. Furthermore, passive diffusion through the swollen tips permits prolonged release of loaded proteins. The reversible swelling/deswelling

property of the MN is exploited to load insulin into the swellable tips through a mild drop/dry method. The MN patch containing insulin was found to release 60% of the insulin over a period of 12 h when exposed to saline, with an estimated 70% of the insulin retaining its structural integrity. A pilot study conducted in vivo showed that the MN patches, which are capable of swelling, resulted in a gradual reduction in blood glucose levels due to prolonged release of insulin [73]. Li et al. have devised an innovative closed-loop MN system, capable of discerning hyperglycemia by detecting glucose levels through environmental alterations catalyzed by the Gox enzyme. This system is energized by a 3.7 V lithium-ion polymer battery and is capable of initiating insulin release from the MNs, rendering it more effective in treating type 1 and advanced type 2 diabetes than other stimuli-responsive MNs. The MN system comprises three principal constituents, namely, an MN glucose sensor, a flexible printed circuit board (FPCB) that performs the function of an integrated recording and control section, and an MN loaded with therapeutics. The FPCB functions as an intermediary between the glucose sensor MN and the therapeutics-loaded MN. The MN glucose sensor employs reverse-iontophoresis to extract interstitial fluid, followed by the detection of glucose levels through GOx-enzyme catalyzed environmental changes. The resultant electric signals are then transmitted to the therapeutics-loaded MN through the FPCB for the iontophoretic release of insulin. All in all, this closed-loop MN system presents itself as an optimal alternative for on-demand insulin delivery in the treatment of type 1 and advanced type 2 diabetes, surpassing other stimuli-responsive MNs [74]. Matsumoto et al. have introduced a novel MN array patch that is enzymatically inert has been synthesized using a semi-interpenetrating network of boronate-containing hydrogel and biocompatible silk fibroin. The incorporation of the boronate-containing hydrogel into the MN array patch enables glucose-responsive diffusion-control of insulin [75]. Phenyl boronic acid-based microneedles (MNs) have been employed for the purpose of glucose-responsive, self-regulated insulin delivery. The fundamental basis of this delivery system rests upon the reversible reaction that occurs between phenyl boronic acid (PBA) and cis-diols. The utilization of PBA, a synthetic Lewis acid, is known to facilitate the reversible binding of hydroxyl groups in glucose, thus leading to cyclic esters formation via the phenyl borate group. This particular reaction serves as the fundamental principle behind the development of the glucose-responsive drug delivery system mediated by PBA [61]. A novel glucose-responsive MN system based on phenylboronic acid (PBA) has recently been

introduced for the experimental treatment of diabetes. In this study, Yu et al. demonstrated the efficacy of a single removable transdermal patch comprising insulin-loaded microneedles and a nondegradable glucose-responsive polymeric matrix fabricated via in situ photopolymerization, in regulating blood glucose levels in insulin-deficient diabetic mice and minipigs. The reversible formation of glucose-boronate complexes between PBA units and glucose under hyperglycemic conditions induced swelling of the polymeric matrix and decreased the electrostatic interactions between negatively charged insulin and polymers, facilitating the rapid release of insulin [76]. Chen et al. have developed a novel core-shell microneedle that is glucose-responsive, utilizing biocompatible materials via a straightforward process. The shell of the microneedle is glucose-responsive and is made up of polyvinyl alcohol (PVA) and ε-polylysine modified with phenylboronic acid, while the core is flexible and composed of pure PVA loaded with insulin. The PVA core not only provides support but also accelerates insulin release. This microneedle has high flexibility and insulin-loading capacity, exhibiting a high puncture efficiency and maintaining its morphology after removal, thus reducing material residues in the skin. Remarkably, the microneedle patch has demonstrated a good hypoglycemic effect in diabetic rats, achieving a hypoglycemic rate similar to that of direct injection after swelling. Moreover, the microneedle patch effectively prevents excess insulin transmission [77]. A novel microneedle patch, which can respond to glucose levels, has been developed to streamline the preparation procedure. The patch consists of a hydrogel composed of phenylboronic acid-grafted polyallylamine and poly (vinyl alcohol) (PVA), cross-linked with boronate ester bonds between phenylboronic acid groups and PVA. The gel retains its fluidity and can be poured into a mold to create microneedle patches, with insulin being directly loaded into the gel. In the presence of glucose, the boronate ester bond is cleaved, leading to a reduction in crosslinking density, resulting in a higher degree of swelling and faster insulin release. The mechanical strength of the microneedle patch is improved through the crystallization of PVA chains via repeated freeze-thaw cycles. The gel exhibits promising glucose-responsive insulin-release functionality, and insulin release can be regulated based on glucose concentration through additional ion crosslinking. In hypoglycemic experiments carried out on diabetic rats, the microneedle patch effectively pierces the skin and slowly releases insulin [78].

Shen and colleagues have elucidated the properties of glucose-responsive MNbased on phenylboronic acid (PBA) that can provide drug delivery in a timely manner. The researchers fabricated a unique hydrogel matrix that not

only elicits drug release in response to glucose but also augments the mechanical integrity of the MNs. The hydrogel system was formulated using *N*-isopropyl acrylamide (NIPAAm), *N*-acryloyl-D-glucosamine (AGA), 3-acrylamide-phenylboronic acid (3-AAPBA), and *N*, *N*′-methylene-bis (acrylamide) (MBA). Upon exposure to elevated glucose levels, phenyl borate ester bonds were formed between PBA derivatives and glucose molecules, thereby reducing hydrophobicity and inducing release of drugs from the hydrogel matrix [79].

4.1.2 Insulin-responsive MNs

Microneedles possess the capability to react to diverse substances, apart from glucose, such as insulin. In response to severe hypoglycemia, Yu et al. have developed a fresh ensemble of insulin-responsive microneedles. This system is fashioned by utilizing insulin aptamers, which comprise of single-stranded oligonucleotides that specifically bond with insulin. Typically, these aptamers affix to immobilized insulin in methacrylate hyaluronic acid (m-HA) microneedles, an exceedingly structurally stable compound that does not discharge insulin. However, in the event of hypoglycemia due to an abundance of insulin, the unbound insulin vies for the aptamer present in the microneedles, thereby triggering the discharge of glucagon and a consequent hypoglycemic reaction. This microneedle system can effectively heighten blood glucose levels by responding to insulin in hypoglycemic conditions, resulting in glucagon release [80].

4.1.3 Electroresponsive MN

The implementation of electrical stimulation in microneedle drug delivery systems is foreseen as an avenue to augment drug delivery through the utilization of electrical signals as an intermediary. This strategy is projected to proficiently amalgamate microneedle drug delivery systems, and there exist diverse manners in which electroresponsive drug delivery can be accomplished. Qi et al. have devised a reversible insulin delivery system that reacts to electricity, utilizing microneedles composed of Thiolated Silk Fibroin (TSF@MNs) that are chemically altered with thiolated silk fibroin (TSF). The addition of graphene (GR) to TSF@MNs to amplify their electrical response allows for the regulation of insulin release by manipulating the degree of swelling in the electrified state, thus resulting in successful glycemic control in individuals with diabetes (Fig. 4A and B). This mechanism provides the opportunity for the manual triggering of insulin administration in the presence of elevated blood glucose levels, thus achieving optimal

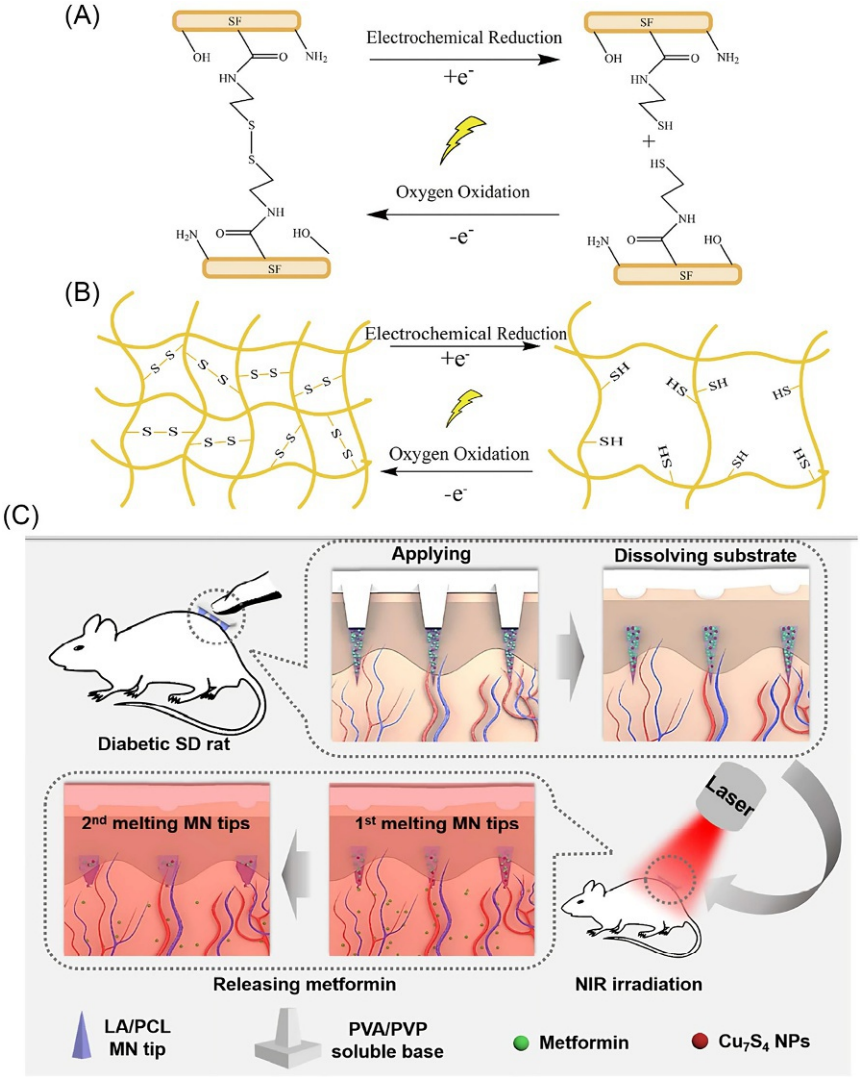

Fig. 4 (A) Reversible change of disulfide bonds-sulfhydryl groups between TSF molecules, (B) disconnection and connection of disulfide bonds between TSF chains [81], (C) NIR triggered transdermal drug delivery on diabetic SD rats [82].

blood glucose regulation. In order to forestall hypoglycemia, the energy source may be expeditiously deactivated upon the restoration of normal blood glucose concentrations, thereby decelerating insulin infusion. The phenomenon of electrification leads to the disintegration of disulfide cross-linking points in TSF, giving rise to the emergence of sulfhydryl

groups that augment the degree of swelling in microneedles and expedite insulin release. The electroresponsive insulin delivery system has been subjected to in vivo experimentation on type 1 diabetic mice, revealing that it can efficiently regulate blood glucose levels before and after meals by means of power supply modulation. This system is capable of sustaining blood glucose levels within the safe interval of 100–200 mg/dL for an extended duration of 11 h [81]. Lee et al. have created a sophisticated system for monitoring and treating diabetes which incorporates various sensors and thermoresponsive MN coated with a layer of phase-change material (PCM) that contains tricyclic acid (TA). The system employs a graphene-based electrochemical patch that activates a heater embedded in the patch when blood glucose levels surpass the predetermined threshold, resulting in the thermal activation of TA and the subsequent release of metformin from the PVP/metformin MNs through a phase transition. In contrast, it was observed that the thermoresponsive MNs remained inactive while the heater was switched off and blood glucose levels were within the normal range. It was further noted that the diabetic mice who were subjected to treatment with thermoresponsive MNs in this state experienced a significant reduction in blood glucose levels, leading to normoglycemic concentration within a span of 3 h. Moreover, the duration of treatment could be prolonged depending on the patient's condition by employing the 4-channel heater array [83].

4.1.4 Light-responsive MN

Light-responsive materials, which are capable of responding to light and are extensively utilized in chemotherapy and photothermal therapy, are produced by integrating photosensitive metal/metal oxide nanoparticles (e.g., LaB_6, Pt, Au, and TiO_2 nanoparticles) or photochromic agents into polymeric microneedles. Near-infrared (NIR) radiation, the most commonly employed electromagnetic wave for activating light-responsive microneedles, is utilized. Light-activated materials can react through a variety of mechanisms. The early group of light-activated microneedles consists of polymers with a low melting temperature that can melt and release their contents [84]. Upon application of near-infrared radiation, the temperature in the proximity of the microneedles rose significantly, reaching 49°C within 10 s and 58°C within 30 s. This notable elevation in temperature resulted in the expedited melting of the microneedles, thereby facilitating drug delivery. Despite their responsive and modifiable drug release capabilities, the high temperatures engendered by the microneedles may be

inappropriate for human tissues. Therefore, it is imperative to identify substances that exhibit lower melting points and to regulate the dosage of NIR, with the ultimate aim of advancing research in this area [85]. Zhang et al. developed microneedles that can be stimulated by near-infrared (NIR) light. These microneedles consist of lauric acid (LA) and polycaprolactone (PCL) tips that include metformin and Cu_7S_4 nanocrystals. When exposed to NIR light, the microneedle tips quickly melt and release the metformin enclosed within, due to the photothermal conversion of Cu_7S_4 and the thermal transformation of LA/PCL (Fig. 4C). By configuring the doses of metformin and Cu_7S_4 nanoparticles embedded in the light-responsive microneedles to 1 mg and 0.1 wt%, respectively, blood glucose levels remained at a normoglycemic level for approximately 4.5 h [82]. Hollow mesoporous silicon dioxide nanoparticles that contain metformin within their structure have been subjected to a process of polydopamine and lauric acid coating. The polydopamine serves the purpose of a photothermal conversion agent, whereas lauric acid functions as a phase-change material. When exposed to near-infrared irradiation, the polydopamine induces the melting of the lauric acid coating layer, which has a melting point of 44°C–46°C. This, in turn, facilitates the opening of the mesopores in silicon dioxide, thereby enabling the drug to diffuse out. When NIR laser irradiation is absent, only a negligible quantity of metformin is released through the drug diffusion route. However, after five cycles of NIR laser on/off, substantial metformin release is observed. This microneedle patch, which is NIR-triggered, exhibits a low risk of hypoglycemia and no in vivo toxicity [86].

Yang et al. have developed microneedles that are capable of responding to both near-infrared (NIR) and electric current stimuli, thereby enabling drug delivery. The scientists have fabricated a microneedle patch composed of polyvinyl alcohol (PVA), which has been coated with a thermally and electrically conductive metal (namely, gold or silver) on its surface. The metal-coated PVA-based microneedle patch has demonstrated a noteworthy 7.9-fold enhancement in in vitro drug release when a DC power supply is applied to deliver 3 V electricity, in contrast to an ordinary PVA-based patch that is devoid of any electrical input. Additionally, it was observed that the microneedles, which were based on polyvinyl alcohol and coated with metal, and were exposed to near-infrared radiation, achieved an approximate temperature of 40°C. This resulted in a 5.3-fold increase in the in vitro release of drugs when compared to a flat patch made of polyvinyl alcohol and not subjected to near-infrared irradiation. On the other hand, the microneedle patch coated with gold showed a relatively low release

of drugs when stimulated by both near-infrared and electric current. The study demonstrated that the developed microneedle patch had a biocompatibility of over 80% in NIH-3T3 fibroblast cells exposed to it [87].

4.1.5 Thermoresponsive MN

The skin temperature exhibits variation within the range of 33°C–37°C across various regions of the human body. Nevertheless, the presence of certain pathological states such as wounds, cancer, or inflammation can lead to perturbations in skin temperature, contingent upon the severity and progression of the disease. This alteration in skin temperature, which is attributed to specific pathological conditions, can potentially serve as a viable approach to facilitate drug delivery through an MN patch [88]. Thermoresponsive MNs that are separable have been produced using a distinct molding technique. These MNs comprise arrowheads that are fashioned out of PCL loaded with metformin on the upper portion and a supportive array composed of PVA/PCL on the lower portion. The arrowheads of the MNs that are loaded with metformin and can be thermally ablated were concealed within a dissolving solid supportive array. Following separation from the substrate, the PCL tips could be effortlessly inserted into the skin. Upon heating the electric heating sheet to 50°C, the PCL tips would undergo a transformation from a solid to a liquid state, thereby discharging the metformin that had been loaded. The method employed in the creation of MNs resulted in remarkable thermal ablation as a result of the low melting point of PCL. Upon insertion into the skin, the upper separable MNs, in the form of arrowheads, were capable of being implanted. Upon exposure to a thermal stimulus, the PCL MNs arrowheads would liquefy, thereby enabling the enclosed metformin to be released in a manner that is thermally modulated. However, it is important to note that prolonged exposure of the skin to elevated temperatures could elicit thermal damage since the transition temperature of PCL (~50°C) is in close proximity to the maximum limit that can be endured by the human body [89].

Li et al. conducted an investigation on the utilization of thermosensitive hydrogel microneedles for the purpose of controlled release of insulin. As the matrix material, gelatin grafted with carboxylic end-capped PNIPAm was employed to fabricate a smart hydrogel microneedle (MN) patch that is crosslinked through physical entanglement. PNIPAm is a thermoresponsive material that has the ability to undergo a sol-gel transition from a linear to a coiled state, which is reversible and is dependent on changes in temperature. In the context of their experimentation, they devised a rapidly separating

MN system by processing GP into RS-GP-MNs, which were mounted onto polylactic acid (PLA) solid MNs coated with polyvinyl alcohol (PVA), for the purpose of controlled insulin delivery. The blood glucose level of the diabetes model mice treated with gelatin-based RS-MNs containing 0.4 IU insulin was significantly reduced within 0.5 h and reached its nadir at 1 h [90].

4.1.6 Mechanical sensitive MN

The application of mechanical force as a stimulus presents an uncomplicated and convenient approach for the spatiotemporally regulated administration of pharmaceuticals. In this regard, mechanical force-sensitive microneedles were utilized for the purpose of dispensing insulin. A flexible microneedle patch was fashioned utilizing an elastomer film comprising of microgel depots that contained nanoparticles (NPs) loaded with the drug. Application of tensile strain expedited the release of insulin from the microgel depots owing to the increased surface area of the depots, as well as the compression exerted on the depots by Poisson's ratio. The present study investigated the in vivo efficacy of insulin-loaded MN in type I diabetic mice. The application of a stretchable MN patch effectively reduced blood glucose levels to a normoglycemic state within 30 min. In clinical practice, stretchable MNs containing micro-depots may be administered on a patient's finger joint to elicit a therapeutic response during finger flexion. This approach enables sustained release during daily activities and intermittent release during intentional movements [24].

Yang and colleagues have successfully devised a novel system for dispensing medication as and when required through the application of mechanical factors. Specifically, the team engineered a touch-actuated MN patch that facilitated the controlled administration of liquid macromolecular drugs. The patch comprises a conventional transdermal patch that had been seamlessly integrated with a solid MN array. By virtue of the patch's unique design, it became possible to regulate dosages through touch-triggered "press-and-release" actions. Upon application of pressure, the patch was able to breach the stratum corneum (SC) layer, which in turn created transient micropores for the purpose of releasing the drug that had been loaded into the patch. The gradual decrease in the blood glucose levels of the rats led to the attainment of the lowest value, which was approximately 152.2 mg/dL. This normoglycemic state was consistently maintained for a duration of 5.2 h. Upon further prolongation of the administration by compression, the normoglycemic state was extended to 7.7 h, while the dose remained

constant. Moreover, the study exhibited that the touch-actuated MN patch possessed remarkable transdermal insulin-delivery capacity both in vitro and in vivo [91].

4.1.7 pH-sensitive MN

Xu and colleagues have tackled the challenge of polymer-based microneedles (MNs) with slow degradation and swelling, which results in prolonged drug release. To address this issue, they have devised a sophisticated pH-responsive transdermal delivery system that regulates the release of insulin sealed internally. This system functions by coating bioactive glass nanopores with ZnO quantum dots. Zinc oxide quantum dots, having a potential as a pH-responsive switch, were employed for capping the nanopores of mesoporous bioactive glasses (MBGs) via electrostatic interaction. The drug, insulin, and glucose-responsive factor, glucose oxidase/catalase (GOx/CAT), were enclosed within the pores of the MBGs. Remarkably, in a diabetic model, this system demonstrated a remarkable hypoglycemic effect, thereby mitigating the risk of hypoglycemia [92]. In the creation of MN-array patches that respond to both H_2O_2 and pH, nanocomplex micelles (NCs) were employed. The distinct characteristic of this framework was the isolation of insulin and glucose oxidase (GOx) in degradable and nondegradable NCs, respectively. This approach ensured the preservation of GOx poststimulation, whereas a particular quantity of insulin was released based on the degree of stimulation. The microneedle matrix gel's core entrapment of the NCs was accompanied by a shielding layer of catalase, an enzyme that scavenges H_2O_2, to safeguard the neighboring tissue from H_2O_2's detrimental effects. Upon an increase in glucose levels, GOx transformed glucose into gluconic acid and H_2O_2, which caused an increase in H_2O_2, disrupting the insulin-NCs and initiating insulin discharge. This approach enabled a more precise and targeted release of insulin in response to specific glucose levels, thereby offering a greater degree of control [93].

4.1.8 Hypoxia-responsive MN

Polymersome-based vesicles were synthesized utilizing a hypoxia/H_2O_2-sensitive diblock copolymer to generate a dual-responsive MN patch for glucose-responsive insulin delivery. The copolymer was affixed with 2-nitroimidazole, a hypoxia-responsive agent, via a thioether moiety, which acted as an H_2O_2-sensitive agent. The polymer underwent self-assembly into a nanoscale bilayer vesicle (polymersome) that enclosed recombinant human insulin and glucose oxidase (GOx) in the aqueous core. When

subjected to elevated blood glucose levels, glucose oxidase (GOx) instigated a reduction in oxygen concentration and an escalation in the levels of reactive oxygen species. Upon attaining the desired concentration of both molecules, the vesicles were prompted to respond by releasing insulin. The vesicle polymer's H_2O_2-sensitive thioether moiety eliminated H_2O_2, thereby enhancing the catalytic function of GOx. In vivo findings demonstrated that blood glucose levels were sustained for a period of 10 h in mice with type I diabetes [94].

4.1.9 Magneto-responsive MN

Zhang and colleagues have successfully designed magneto-responsive MN robots that are capable of delivering insulin to the intestines. These robots are comprised of three essential components, namely, an insulin-laden tip, a separable connection, and a magnetic substrate. The MN robots were affixed to capsules that could degrade in the intestinal environment, thereby facilitating the delivery of insulin. Upon administration via the oral route, the micro-robotic systems known as MN robots, which were present in the intestine and poised for release, were directed through the intestinal tissue by means of an externally applied magnetic field. This allowed for sufficient penetration of the intestinal barrier. Subsequent dissolution of the MN tips facilitated delivery of insulin to the target site. Following this process, the magnetic substrate was eliminated from the organism. Remarkably, the MN robotic systems demonstrated effective regulation of blood glucose levels in pigs with STZ-induced diabetes, comparable to that observed in healthy pigs following glucose consumption [95].

4.2 Cancer

Changes in pH in various organs, or the presence of low pH at sites of chronic wounds, inflammation, and cancer, have been extensively investigated as triggers for the development of pH-sensitive drug delivery systems [96,97]. Since the microenvironment of most tumors has a lower pH than noncancerous tissue, pH-sensitive properties have practical implications for cancer therapy. pH-responsive carriers can be incorporated into MNs to target and control the delivery of therapeutic agents to the skin by the acidic environment of the epidermis. Additionally, pH-sensitive drug delivery systems can be used for targeted delivery of antitumor therapeutics [98,99]. A microneedle patch utilizing pH-responsive hollow polylactic glycolic acid (PLGA) microspheres has been developed for transdermal drug delivery. The model drug used in this study was cyanine 5, delivered to the skin

via polyvinylpyrrolidone (PVP) microneedles. PLGA hollow microspheres (HM) containing sodium bicarbonate ($NaHCO_3$) encapsulated cyanine 5. The epidermis has an acidic environment with a pH of about 5.5 and is the layer where skin cancer usually occurs. The acidic environment of the epidermis stimulates PLGA-HM containing $NaHCO_3$ to form CO_2 bubbles, forming pores in the PLGA envelope and rapidly releasing the model drug [100]. Li et al. have developed pH-responsive coated MN for the effective administration of p53 DNA in the treatment of skin cancer. The study has employed the cutaneous microenvironment pH, which is estimated to be 5.5, to facilitate the rapid release of p53 DNA. The microneedles have been engineered with a polycaprolactone (PCL) core, which has been coated with a pH-responsive polyelectrolyte multilayer comprising of dimethyl maleic anhydride-modified polylysine (PLL-DMA) and p53 DNA using a layer-by-layer assembly strategy. Upon encountering the acidic milieu of the skin, the negatively charged PLL-DMA coating on the MN undergoes a transformation into positively charged PLL, thereby triggering the dissociation of the coated layer and accelerating the rapid release of p53 DNA. The PLL-DMA-coated MNs evince a 33% discharge of p53 DNA in a buffer solution of pH 5.5, while the MNs devoid of PLL-DMA manifest only a 4% release. Additionally, the in vivo analysis on a tumor-bearing mice model substantiates that the PLL-DMA-coated MNs exhibit an efficacy of 90.1% in inhibiting tumor growth. Conversely, the mice that were treated with non-PLLDMA MNs and those that received intravenous administration displayed only 46.4% and 30.5% tumor inhibition efficacy, respectively [66].

In recent years, there has been a significant focus on researching immunotherapies for the treatment of skin cancer. Notably, checkpoint inhibitors that hinder the programmed death-1 (PD-1) pathway have displayed remarkable clinical efficacy in various studies. Phase II and III clinical trials for advanced melanoma have indicated that anti-PD-1 antibodies that target the inhibitory receptor have exhibited substantial antitumor activity. Nevertheless, cancer immunotherapy is faced with various challenges, including the enhancement of response rates and the surmounting of drug resistance. Wang and colleagues have successfully crafted a hyaluronic acid-based MN patch with a physiologically self-degradable property, which is fortified with pH-sensitive dextran nanoparticles. The primary goal of this innovative technology is to support cancer immunotherapy by enabling controlled delivery of aPD1 to melanoma. The pH-sensitive dextran nanoparticles were generated through a meticulous double emulsion (water-in-oil-in-

water) solvent evaporation/extraction technique, which was complemented by a GOx/CAT enzymatic system and aPD1. The dextran nanoparticles that are sensitive to pH were incorporated into the MN, which is based on polymers. A highly acidic environment was created to promote the self-dissociation of the NPs, resulting in a significant release of aPD1. In vivo experiments conducted on mouse models with melanoma demonstrated that a single application of the MN patch was more effective in inhibiting tumor growth than intratumoral injection (i.t.) of the same dose [101].

Lan et al. conducted a study that investigated the application of pH-responsive tumor-targeted lipid nanoparticles-loaded MN in the facilitation of synergistic immuno-chemotherapy for the treatment of cancers. The MNs were engineered to deliver both antiprogrammed death-1 (aPD-1) antibody and cisplatin in a pH-responsive manner. This was achieved by integrating lipid-coated cisplatin nanoparticles and aPD-1 and CDDP (cis-diammineplatinum(II) dichloride) into the PVP solution which was then cast into a mold. The pH-responsive MNs exhibited improved efficacy toward tumor cells in the immunocompetent murine tumor homograft model and a reduction in systemic toxicity of cisplatin, which is indicative of their potential as a promising approach to cancer treatment [102]. Ye and colleagues have formulated a novel approach for the transcutaneous delivery of melanoma treatment, utilizing microneedles. This approach is based on a synergistic immunotherapy strategy that targets the immunoinhibitory receptor programmed cell death protein 1 (PD1) and the immunosuppressive enzyme indoleamine 2,3-dioxygenase (IDO) at a localized level. The immunotherapeutic nanocapsule has been designed to contain anti-PD1 antibody (aPD1) and is constructed from hyaluronic acid that has been modified with 1-methyl-DL-tryptophan (1-MT), which acts as an IDO inhibitor. This particular formulation method, which involves combining "drug A in carriers formed by incorporation of drug B," has been shown to enhance the capacity for loading therapeutics. Subsequently, the release of aPD1 is triggered, which effectively blocks PD1 in the context of an increased population of alloreactive T cells. Through the use of a B16F10 mouse melanoma model, it has been demonstrated that this synergistic treatment has achieved potent antitumor efficacy [103].

Light-responsive MNs are often utilized in a combined approach involving drug-loaded MNs and photothermal therapy. Photothermal therapy operates by converting light to heat, thereby causing cellular damage and tumor eradication, while simultaneously triggering the release of anticancer drugs embedded within the MNs. This synergistic treatment modality

exhibits promising outcomes for antitumor therapy, as it enhances drug delivery efficacy and augments therapeutic effects. The utilization of light-responsive MNs represents a burgeoning field of investigation and possesses the potential to revolutionize the management of various types of cancer. Research has shown that the synergistic application of photothermal therapy and chemotherapy can be a highly efficacious means of treating malignant neoplasms. This method offers the potential for a decrease in drug dosages, which can help to reduce the unfavorable effects that often accompany traditional single-agent treatments [52]. Chen and their research team endeavored to accomplish the aforementioned goal through the development of a transdermal drug delivery system intended for cancer treatment. The team commenced by fabricating nanostructures, which they christened NIR-sensitive silica-coated lanthanum hexaboride (LaB6@SiO2). Subsequently, this innovative nanostructure was integrated into PCL-based MNtogether with DOX. Upon exposure to NIR irradiation at 808 nm, the LaB6@SiO2, located within the PCL-MNs, generates heat, thereby causing the PCL to dissolve, consequently releasing the DOX from the MNs. The microneedles (MNs) exhibited melting behavior at a temperature of 50°C, within a span of 80 s of being subjected to NIR irradiation, and underwent complete melting after a duration of 10 min. Furthermore, the MNs showcased the ability to release drugs in a switchable manner, with drug release being initiated and terminated via NIR irradiation for a minimum of three cycles. Upon NIR irradiation exposure, the use of LaB_6@SiO_2-loaded MNs in rat experiments led to the localization of DOX at a depth of 300 μm in the skin [104]. Dong and colleagues have successfully produced MN arrays composed of hyaluronic acid that have been impregnated with gold nanocages (AuNCs) and the chemotherapeutic agent doxorubicin (DOX). The utilization of AuNCs not only augmented the efficacy of the photothermal therapy, but also contributed to the MNs' mechanical robustness. These MNs have demonstrated the ability to puncture the skin, dissolve within the tumor site, and release the pharmaceutical agents with remarkable efficiency. The combination of the photothermal effect of the AuNCs following near-infrared laser irradiation and the chemotherapy effect of DOX worked synergistically to destroy tumors. The researchers administered DOX/AuNC-loaded MNs to mice with subcutaneous soft tissue tumors and observed a notable antitumor effect after four administrations, with no apparent side effects [105].

Ye et al. have proposed a novel strategy for cancer immunotherapy that integrates melanin and light-activated microneedles (MNs). The researchers

loaded B16F10 whole tumor lysate containing melanin into hyaluronic acid-based MNs. These MNs enabled the gradual release of the lysate upon insertion into the skin. When the combination of MNs and near-infrared light irradiation is employed, the melanin produces heat, thereby intensifying the uptake of tumor antigens by dendritic cells and consequently resulting in an improved antitumor vaccination. Following the administration of synthetic melanin-loaded MNs with NIR treatment to mice afflicted with tumors, the results indicated that there was an 87% and 37% reduction in melanoma and 4 T1 carcinoma tumors, respectively, within a span of 30 days [106]. Hao et al. have devised a method whereby NIR light-responsive MNs are employed to augment the antitumor effect. The MNs are composed of a PEG-plated gold nanorod (GNR-PEG)-coated array and docetaxel (DTX)-loaded micelles. The process is executed in two stages: first, via tail vein injection of the DTX-loaded micelles, and second, by inserting the MNs into the tumor site. Each MN, containing a mere 31.83 μg of GNR-PEG, demonstrated exceptional heating performance, thereby enhancing the antitumor efficiency of low-dosage DTX-loaded micelles (5 mg/kg). The tumors of the treated mice decreased rapidly and were ultimately healed within 14 days [107]. Wang et al. established an MN patch that is light-responsive and loaded with doxorubicin (DOX) for the purpose of chemo-thermal therapy of superficial malignant tumors in their research. The authors employed the use of Flav7, an organic molecular fluorophore, as a photothermal agent, which was blended directly with the PCL matrix to prepare the MN patch. In vitro investigations have demonstrated that the MN patch is capable of being activated and deactivated for a minimum of five cycles, with no instances of drug leakage during the dormant state. When utilizing a 4T1 breast tumor-induced mice model in experimentation, administering Flav7 and DOX-loaded MNs resulted in the total eradication of tumors in contrast to using solely Flav7-loaded MNs. Additionally, all subjects survived for 50 days without any signs of tumor recurrence [108].

Alvaro et al. have fabricated MN arrays and individual needles using cross-linked polymers and coated with gold nanorods (GNRs). The GNRs exhibit heat generation upon near-infrared light absorption, attributable to the phenomenon of localized surface plasmon resonance. The use of MNs in GNR delivery precludes the occurrence of side effects, including metal deposition and metabolism. Upon insertion of the MNs patch into the skin and subsequent exposure to near-infrared light, high heat is generated in a short duration (up to 15.95°C in 8 s), potentially enabling the treatment of

deep skin hyperthermia [109]. The research conducted by Sivaraman and Banga centers on the utilization of in situ-forming hydrogel MNto deliver drugs transdermally, with the normal cutaneous temperature range of 32°C–37°C serving as a stimulus. The authors implemented the conventional "poke and patch" approach, wherein solid MNs were first administered onto the skin surface to generate micropores, followed by the application of a drug formulation for transdermal delivery. However, rather than utilizing conventional hydrogel topical formulations, an in-situ forming hydrogel solution that undergoes a sol-gel transition at the skin's temperature was employed subsequent to the insertion of microneedles (MNs). The temperature responsiveness of the in situ-forming hydrogel MNs, which plays a crucial role, is attributed to the presence of poloxamer. In vitro studies indicated that the use of poloxamer-based in situ-forming hydrogel MNs significantly improved the permeation of methotrexate (a model drug) compared to non-in situ-forming (non-poloxamer-based) formulations. Moreover, the in situ-forming hydrogel MNs exhibited sustained methotrexate release for a period of 72 h, whereas the non-in situ-forming formulation achieved methotrexate saturation within 35 h [110].

4.3 Wound healing

Chronic wounds, such as diabetic wounds, tumor wounds, and pressure sores, that are caused by bacterial infection pose a significant medical challenge. The inflammatory response is a crucial aspect of wound healing and begins as soon as the wound occurs [111]. In comparison, an extended period of inflammatory response can lead to a delay in the healing process and the formation of scars. On the other hand, a moderate inflammatory response is imperative for the effective clearance of infected tissue and the efficient restoration of affected areas. Researchers have employed the efficacious delivery capability of MN to devise diverse antiinflammatory approaches. MNs have demonstrated substantial reduction in levels of inflammatory factors by enclosing active ingredients such as antiinflammatory drugs and antibodies. Furthermore, MNs have been discovered to not only augment permeability but also provide sturdy stability of the enclosed substance, thereby facilitating sustained and regulated release. This attribute is imperative in mitigating drug resistance [112]. Since bacterial biofilm formation is almost always involved in chronic wounds in humans, antibiotics are often used to accelerate the wound healing process. Stimuli-responsive microneedle patches have been developed for the efficient management of

wounds by delivering antibiotics and angiogenic agents, utilizing the wound microenvironment such as pH and temperature as a stimulus [56]. One example of stimuli-responsive microneedles is an array of chitosan-graphene assembled in porous carbon (CS-GAPC) nanocomposites that have been synthesized and evaluated. The efficacy of this system has been tested by evaluating the drug delivery properties initiated by stimuli such as pH and electric field. The drug cephalexin was incorporated into these MNs. The prepared nanocomposite showed pH-dependent drug release, with complete release of the encapsulated drug at an acidic pH of 4. Optimum drug release was achieved with an electric field of 5 V, as a function of applied electric pulses [113].

Ullah and colleagues have successfully engineered an MN patch that is coated with a pH-responsive material to achieve precise and targeted delivery of antibiotics to the affected region through a "closed-loop" methodology. The team utilized the alkaline pH levels in the wound site as a biomarker to ensure accurate administration of medication. The MN system is composed of a stainless steel core, which is encased with PLGA polymer in order to generate pores that are capable of holding the drug. Upon the drug of interest being loaded, the pores were subsequently sealed with a thin layer of Eudragit S100 to hinder exposure to the embedded drug. In an acidic environment, the in vitro investigation exhibited minimal drug release at pH 4.5 owing to the insoluble Eudragit S100 layer. The thin Eudragit S100 layer facilitated rapid dissolution of the MN patch, resulting in the release of 76% of the drug at pH 7.5. The in vivo study revealed an absence of fluorescence signal for healthy skin, connoting nonrelease of the drug at acidic pH. Conversely, the wounded skin region demonstrated significant fluorescence signal, indicating the alkaline pH-responsive nature of drug release from the MN system [114]. A recent investigation has explicated the creation of an MN system that is responsive to bacteria, designed to manage chronic wounds that have become infected. The aforementioned system incorporates silver nanoparticles (AgNPs) into microparticles (MPs) that are responsive to bacteria, which are composed of poly (ε-caprolactone) adorned with chitosan, and these are subsequently loaded into dissolving MNs fashioned from PVA/PVP. Silver nanoparticles (AgNPs) were employed as an antibacterial agent, and a lipolytic esterase enzyme was employed as a stimulus for the delivery of the antibacterial agent by dissociating the polycaprolactone-chitosan (PCL-CS) microneedle patches (MNs). The MNs treated ex vivo rat skin biofilm model exhibited a complete reduction of 100% in bacterial bioburden. Conversely, conventional

cream loaded with AgNP@PCL-CS-MPs and free AgNPs-loaded MNs merely eradicated 60% and 75% of the biofilm formed by *Staphylococcus aureus* and *Pseudomonas aeruginosa* strains, respectively [115].

Mir and their team have employed bacterial enzymes as a stimulus for wound management, which is consistent with prior research. Specifically, they have created bacteria-responsive MNs that are capable of administering carvacrol (CAR), a natural antibacterial agent, to the affected area for optimal wound healing. To achieve this goal, the authors have harnessed the lipolytic esterase enzyme secreted by *S. aureus* and *P. aeruginosa* to facilitate the release of the encapsulated antibiotics from the MN patch. In the beginning, there was a development of CAR-encapsulated poly (ε-caprolactone) nanoparticles (PCL-NPs) which were subsequently loaded into poly (vinyl alcohol)/polyvinyl pyrrolidone (PVA/PVP)-based dissolving MNs. Upon contact with the lipolytic esterase enzyme, the PCL-NPs can dissociate and thereby release CAR. The in vitro findings demonstrated that 72% of CAR was released from the PCL-NPs in the presence of *S. aureus* and *P. aeruginosa* strains, while only 17% of CAR was released from the same NPs in the absence of bacterial strains [116]. Su and colleagues have recently presented a novel microneedle patch that responds to near-infrared (NIR) light, facilitating the controlled release of antimicrobial peptide for the treatment of wound biofilms. The microneedle patch comprises IR780 iodide, which converts light to heat, and W379 peptide, a potent antimicrobial agent. To ensure effective delivery of the therapeutic agents, these constituents are incorporated into dissolvable poly (vinylpyrrolidone) (PVP) microneedles that are coated with 1-tetradecane (TD), a phase change material. Upon exposure to near-infrared radiation (NIR), the IR780 molecule induces thermal degradation (TD), resulting in solubilization of the polyvinylpyrrolidone (PVP) microneedles and consequent release of the W379 peptide into the adjacent milieu. This innovative microneedle transdermal patch is endowed with a predetermined peptide release profile and exhibits remarkable antimicrobial activity against various bacterial strains in vitro. Furthermore, the patch demonstrates exceptional antibiofilm efficacy in ex vivo and in vivo settings, thus showcasing its immense potential as a versatile platform for delivering various other antimicrobial agents [117]. Sun and colleagues recently conducted a study regarding light-responsive microneedles (MNs)-based liquid band-aids and their effectiveness in wound healing. The system was developed by integrating porphyrin-like metal-center nanoparticles (PMCS) into PVA-based MNs. PMCS serves as the primary component of the MN system, capable of generating heat upon exposure to

NIR irradiation at 808 nm. The heat produced by PMCS facilitates the transformation of MNs from their solid state to liquid state, thus enabling complete coverage of the wound. The MN containing PMCS that have been liquefied will undergo solidification again in the absence of NIR irradiation due to the cooling effect. The enzyme-like activity of PMCS resulted in the successful destruction of *S. aureus* by generating ROS and high temperature under NIR irradiation. The cytotoxicity of PMCS toward NIH-3 T3 fibroblast cells at a concentration of 100 μg/mL was insignificant, with more than 85% cell viability. Furthermore, it is noteworthy to mention that the rate of hemolysis was found to be less than 5%, which is indicative of the exemplary biocompatibility of PMCS. Moreover, the utilization of PMCS-loaded MNs with NIR resulted in a remarkable 86.6% wound healing within a mere 7 day period, as observed in a mouse model with wounds [118].

Yao et al. conducted a study on MNs that are responsive to near-infrared (NIR) radiation and made of metal-organic framework (MOF) for the purpose of delivering nitric oxide (NO) into the wound bed of diabetic rats. The developed MOF-MNs effectively facilitated the wound healing process in diabetic rats. The NO-loadable copper-benzene-1,3,5-tricarboxylate (HKUST-1) MOF is encapsulated with graphene oxide (GO), resulting in microparticles known as NO@HKUST-1@GO (NHGs), which possess a photothermal response feature in the NIR region and enable the controlled release of NO molecules. When NO@HKUST-1@GO microparticles (NHGs) are incorporated within a porous PEGDA-MN, the resulting porous structure, enlarged specific surface area, and adequate mechanical strength of the integrated MN may facilitate a more precise and profound delivery of NO molecules at the wound site. The authors showcased the potential of NHG-MN in wound healing and other therapeutic applications by applying it to the wound of a type I diabetic rat model, which resulted in the acceleration of vascularization, tissue regeneration, and collagen deposition [119]. Sun and colleagues have introduced a novel approach for wound healing utilizing an adenosine-coated, MXene-integrated MN patch. The formation of the MN host was achieved through crosslinking 3-(acrylamido) phenylboronic acid-(PBA-) and PEGDA employing the use of UV light. MXene was incorporated into the interior of the MNs, while adenosine was coated on the exterior of the MNs via a soaking process. Under infrared irradiation, the MXene generates heat, thereby facilitating the release of adenosine. As an active agent in the body, adenosine can stimulate skin repair, neovascularization, and fibrosis [120]. Recent

investigation has presented novel photothermal and temperature-sensitive microneedle patches that may be activated by near-infrared (NIR) light. The research team produced Fe3O4-loaded gelatin-PEGDA hydrogel (Fe3O4-GP) and Fe3O4-GP microneedle (Fe3O4@MN) by straightforwardly polymerizing gelatin, Fe3O4 nanoparticles, and poly (ethylene glycol) diacrylate (PEGDA) monomer. By integrating gelatin into the material system, the microneedle becomes temperature-sensitive and collaborates with the photothermal response of Fe3O4 nanoparticles under NIR irradiation. The Fe3O4@MN composite material exhibits sufficient mechanical robustness to puncture the skin barrier, and when subjected to NIR irradiation, the temperature of the Fe3O4@MN patch can be elevated by 40°C within a minute. In vitro experimentation revealed that the rate of drug release from the Fe3O4@MN patch was 80% after 20 min, while the release rate of doxycycline hydrochloride from Fe3O4@MN was 70% after 20 min of NIR irradiation. Subsequent penetration studies demonstrated that the skin permeation depth of model drugs transported by Fe3O4@MN patches under NIR irradiation was 150–200 lm greater than that of patches without Fe3O4 nanoparticles [121].

Guo and colleagues have developed an MN patch that is temperature-responsive and draws its inspiration from shark teeth. The MNs are fashioned in a shark tooth-like (inclined) design, with the objective of ensuring strong adhesiveness to the skin. The MN patch is flexible and has been created using silk fibroin (to provide rigidity), polyurethane (to provide flexibility), and NIPAAm (to provide temperature responsiveness). The wound areas can often exhibit a temperature increase of up to 39.4°C due to severe infection. This temperature increase can result in the MNs contracting and discharging embedded drugs into the wounded area, making the patch suitable for intelligent wound management. The results of the in vitro investigation indicated that the MNs exhibit effective drug release at 40°C, coupled with a subsequent contraction and expansion to their initial state at ambient temperature. Additionally, it was observed that the MN patch containing fluorescein was stable for a period of 7 days at ambient temperature, with negligible loss of fluorescein. This outcome highlights the potential of the MNs for storage stability. To further validate the resilience of the MNs in a biological fluid environment, the MN patch was submerged in a phosphate buffer with a pH of 7.4 at 25°C for a duration of 24 h [122]. Chi and his team utilized a thermo-responsive MN system to stimulate wound healing. The MN system comprises a chitosan (CS) hydrogel that is combined with VEGF-encapsulated poly (NIPAAm) gel and subsequently subjected to

UV polymerization. The NIPAM component of the system allows for VEGF release only when the temperature of the wound increases due to inflammation, resulting in efficacious wound healing. Additionally, the MNs exhibited a 99% lethality rate against both Gram-positive (*S. aureus*) and Gram-negative bacteria (*Escherichia coli*). Furthermore, VEGF-loaded CS MNs facilitated optimal wound healing in Sprague-Dawley rats within a span of 9 days, surpassing the results of plain CS MNs and CS films [123]. A notable approach for addressing biofilm infection involves a drug delivery system using polymer micelle nanoparticles. Researchers have developed a biodegradable MN patch that can deliver drugs. The microneedles are equipped with micelles formed by a pH-responsive tri-block polymer (DMA-PEI-PLGA, DPP) and an antimicrobial peptide coupled with Ce6 (DPP@AC). The microneedle's tip consists of a hydrogel called HAMA, loaded with DPP@AC, which is also used as the support structure's second layer. In laboratory experiments, it was discovered that the utilization of DPP@AC microneedles resulted in the inhibition of 90% of biofilm formation and exhibited a remarkable MIC90 value of less than 5 μM against *S. aureus* when exposed to laser irradiation. An in vivo experiment was then conducted by the researchers where the microneedles were inserted into the skin of diabetic mice infected with *S. aureus* biofilm. Within a mere 10 min of insertion, the microneedles were found to be degraded. Subsequently, the microneedle patch, which was loaded with DPP@AC, was applied to the wound and exposed to laser irradiation, which resulted in a significant antibacterial effect due to the synergistic action of the antimicrobial peptide and photodynamic therapy. Furthermore, it was observed that the biodegradable microneedle patch was capable of responding to the acidic environment in the inflammatory environment, thereby leading to the rapid and effective release of antimicrobial agents [124].

In recent years, there has been a growing interest in utilizing antimicrobial photodynamic therapy as a novel approach to combat microbial infections. This innovative therapy utilizes a photosensitizer that generates reactive oxygen species (ROS) or reactive transients when exposed to light of a specific wavelength, which typically falls within the range of 400–700 nm. ROS-responsive MNs have demonstrated significant potential for addressing inflammation. For instance, researchers have loaded clindamycin, an antibiotic, into ROS-responsive polymeric MNs. These MNs can effectively penetrate the skin's outer layer and deliver the drug to the targeted site of inflammation within the designated therapeutic range [125]. ROS-responsive MNs have been employed for the topical treatment

of inflammation induced by *Propionibacterium acnes*, a Gram-positive human skin commensal that contributes to the pathogenesis of acne. Despite the progress made in dermal and oral administration of antiacne medication, managing these cutaneous lesions still presents certain challenges. These limitations arise from the inadequate penetration of antibiotics through the stratum corneum and the potential deleterious effects associated with high doses of antibiotics [126,127]. Zafar et al. developed microneedle patches that are capable of dissolving in response to pH changes, with the aim of treating biofilms. These patches consist of microneedles composed of hyaluronic acid and sorbitol (CP), as well as clarithromycin microfibers coated on microneedle patches (MP). The formulation used in this study included PVP at a concentration of 25% *w/v*, and HA at a concentration of 1.5% w/v, combined with sorbitol (10% *w*/w polymer) as a plasticizer. Upon application to the skin, the formulations demonstrated rapid uptake of physiological fluid, with $96.0 \pm 0.92\%$ swelling within 15 min. The formulations underwent assessment for clarithromycin release profile in vitro, antibacterial activity against specific bacterial strains, and in vivo antibiofilm activity against *S. aureus* in albino rats. The dissolving microneedle patches based on sorbitol displayed a drug release of approximately 82%, while the MP facilitated an alkaline pH (7.4) responsive drug release of approximately 81% within 2 h. Moreover, the MP exhibited an inhibitory zone approximately 13% larger against *S. aureus* in comparison to CP [128].

RNA-based therapeutic modalities present a promising avenue for the treatment of infectious diseases. Nonetheless, the physicochemical attributes of RNA molecules, including their vulnerability to degradation mediated by nucleases, constitute a significant hindrance to their use as therapeutic agents for such diseases. To surmount this challenge, a GO-based transformation system for ASyycF delivery that functions as an antimicrobial agent has been developed. Moreover, Wu et al. have devised a core/shell microneedle patch that comprises of hyaluronic acid methacrylate (HAMA), carboxymethyl chitosan (CMCS), and sodium alginate, which is responsive to enzymes. This microneedle patch has been expressly designed for the treatment of chronic diabetic wounds. The microneedle patch designed for the treatment of chronic diabetic wounds features a moisture layer that serves to uphold the moisture balance in the wound exudate. The microneedles are secured in position by means of a chitosan adhesive layer and possess ample strength to penetrate the biofilm, eliciting dispersion. The microneedle's HAMA shell undergoes degradation, thereby releasing graphene oxide with ASyycF, which triggers potent antibacterial activity. Additionally, the patch

dispenses basic fibroblast growth factors from the CMCS core, which accelerates angiogenesis, collagen synthesis, and immunity modulation. This multifaceted microneedle patch holds significant potential for expediting the healing of infected diabetic wounds [129]. Psoriasis is a form of skin disease that involves inflammation, and there exists potential for MN patches to enhance treatment outcomes by increasing the concentration of local medication in the skin. Given the tendency for psoriasis to recur, it is vital to develop intelligent MN-based drug delivery systems that can maintain therapeutic drug levels over extended periods and improve treatment efficacy. To address this need, Bi and colleagues have developed detachable gel-based MN patches that are responsive to H_2O_2 and contain both methotrexate (MTX) and epigallocatechin gallate (EGCG). The purpose of using EGCG as a cross linker in this context is to extend the duration of psoriasis treatment. The elevated loading capacity observed in EGCG within the gel needle tips can be attributed to the hydrogen bonding interactions between EGCG and phenylboronic acid-modified hyaluronic acid. Upon insertion into psoriasis-like skin, the gel needle tips undergo separation from the supporting arrays and subsequently swell into porous gels following contact with the interstitial skin fluid. These porous gels serve as drug reservoirs, enabling controlled drug release with MTX being diffusively released from the porous needle tips to provide prompt treatment for psoriasis. ROS and acidic pH serve as triggers for the release of EGCG from the gel needle tips within psoriatic skin. Notably, the cross-linked gel needle tips exhibit a skin residence time of approximately 4 days, leading to a more sustained release of EGCG [130]. Yang et al. have produced a transdermal delivery system aimed at addressing psoriasis by utilizing a self-powered electroresponsive MN patch. The patch incorporates a piezoelectric nano generator (PENG) as a means of generating an electric current, which is then directly connected to a Dex-loaded electroresponsive MN patch. Comprising of two sets of microneedles (MNs), the MN patch consists of a working electrode and a counter electrode. The MNs utilized as the working electrode were fabricated by employing a combination of polylactic acid-gold-polypyrrole (PLA-Au-PPy) and Dex, whereas the counter electrode MNs were composed of polylactic acid-gold (PLA-Au). The electroresponsive material polypyrrole, which is present in the working electrode MNs, can transition between the oxidative and reductive states when subjected to electric stimulation, thereby leading to the liberation of Dex from the MN system. Notably, in a rat model, the PENG-integrated MN patch was found to exhibit superior drug release in comparison to conventional battery-integrated

MNs. The results obtained from an in vitro biocompatibility study conducted on RAW 267.4 cells demonstrated that the developed MNs displayed a cell viability of over 85% [131].

Xiang et al. have recently published a study describing a microneedle patch composed of sodium hyaluronate. This patch has the ability to facilitate transdermal delivery of ultrasound-responsive nanoparticles, which can effectively treat *P. acnes*. The authors utilized a composite structure consisting of zinc porphyrin-based MOF and zinc oxide (ZnTCPP@ZnO) and incorporated it into the MNs synthesized from sodium hyaluronate. Upon application of the MNs onto the skin, they dissolve into the dermis and subsequently release the nanoparticles. The application of composite materials has a profound impact on sonocatalytic performance, as it enhances interfacial charge transfer and diminishes the energy required for oxygen activation. When subjected to ultrasound, oxygen undergoes electron gain, transitioning from a ground state to an excited state and generating an abundance of ROS that effectively eliminates *P. acnes*, thereby treating acne. Furthermore, the release of zinc ions from the nanomaterial leads to the upregulation of metallothioneins (Mt) 1 and Mt2 in skin fibroblasts. The results of the study demonstrate that this activated oxygen-mediated approach yields an antibacterial efficiency of 99.73% against *P. acnes* within a 15-min ultrasound irradiation period, resulting in a decrease of acne-related factors such as tumor necrosis factor-α, interleukins, and matrix metalloproteinases [132]. The team headed by Zhen has developed a microneedle patch that exhibits a high degree of sensitivity toward reactive oxygen species (ROS) and has the capacity to specifically target inflammatory acne. The patch incorporates an antibiotic that is incorporated into a double cross-linked ROS-responsive joint, which is created by polyvinyl alcohol (PVA) and phenylboronic acid. Upon application of the patch to the surface of inflamed acne skin, the ROS disrupts the cross-linked structure of the hydrogel microneedles, leading to the dissolution of the needle tip and release of the antibiotic [133]. Acne vulgaris is a medical condition that is characterized by an increase in the levels of reactive oxygen species (ROS). These species serve as a significant stimulus for the precise delivery of antibiotics to the target region. Under pathological conditions, inflammatory tissue may exhibit ROS levels that exceed 500 μm, whereas normal tissue typically ranges between 1 and 15 μm. Zhang et al. capitalized on this concept and developed a ROS-responsive MN patch that can deliver CDM exclusively to the site of *P. acnes*-infected follicles in a controlled manner using an on-demand strategy. First, an experimental approach involving

the cross-linking of polyvinyl alcohol (PVA) with a dual phenylboronic acid derivative was employed. Subsequently, the addition of chlorhexidine digluconate (CDM) facilitated the creation of a CDM-loaded ROS-responsive gel, denoted as CDM@RR gel. Through in vitro testing, it was observed that the developed MN possessed a faster rate of CDM release in the presence of *P. acnes* and hydrogen peroxide. Conversely, no drug release was observed in the absence of *P. acnes* and H_2O_2, thereby affirming the ROS-triggered drug release mechanism. This feature yields an on-demand treatment effect for acne inflammation. Notably, the CDM@RR MNs-treated group displayed complete recovery of inflammatory lesions within 6 days without any adverse effects on neighboring skin tissue [134].

Androgenetic alopecia (AGA) is a prevalent type of nonscarring alopecia that is both progressive and hereditary, characterized by the miniaturization of terminal hair into fine, vellus hair. Recent studies have focused on the effects of micro-needling on hair regrowth in individuals with AGA, after observing hair growth in mice due to micro-needling. In their research, Fang and colleagues have introduced a magneto-responsive MN patch that utilizes an externally applied magnetic field to achieve controlled drug release for the treatment of AGA. The investigators initially synthesized mesoporous iron oxide nanoparticles through the encapsulation of minoxidil (Mx), and subsequently incorporated the Mx-MIOs into PVA-based microneedles (Mx-MIOs@MNs). The microneedle patch was designed to be applied on the area of interest for hair growth, followed by the application of a magnetic field to facilitate the controlled release of the drug from the microneedle patch. Upon exposure to an external magnetic field, the Mx-MIOs induce a rise in temperature within the MNs as a result of the magneto-responsive characteristic of iron oxide, thereby enabling the release of the encapsulated Mx at the targeted site. In vitro investigations have demonstrated that Mx release from MNs reached 20% after a period of 120 min without the influence of a magnetic field. However, when a magnetic field was applied for a duration of 1 min, more than 40% of Mx was released from the MNs within the same time frame. The duration of the magnetic field application was further found to have a positive correlation with the enhanced drug release within the desired time frame. Nonetheless, the Mx-MIOs@MNs + magnetic field for 1 min (enabling a temperature of less than 40°C) has exhibited relative cytotoxicity toward HIG-82 fibroblast cells at a concentration of 200 mg/mL, with more than 75% cell viability [135]. The topic of delivering therapeutic agents that enhance the local concentration of active molecules has garnered significant

attention in numerous biomedical applications. Ullah and colleagues have detailed the development of intelligent microneedles featuring porous polymer coatings for pH-responsive release of lidocaine, a local anesthetic. This microneedle system comprises stainless steel needles coated with poly (lactic-*co*-glycolic acid) (PLGA) polymer, which results in the creation of a porous structure on the surface of the smart microneedles (SSMNs). Lidocaine, a representative drug, is utilized in conjunction with $NaHCO_3$, a pH-sensitive material, to load the model drug. The pores on the MNs are then capped and sealed using a delicate layer of PLGA. Subsequent exposure to an acidic environment results in the generation of CO_2 from $NaHCO_3$ present within the pores. This, in turn, leads to the disruption of the thin PLGA layer and the release of the drug at an acidic pH. In vitro studies have demonstrated that, by the end of 60 min, approximately 98% of lidocaine is released from the MNs at pH 5.5, while, at pH 7.5, lidocaine release does not exceed 20% [136].

4.4 Vaccine delivery

The administration of preventive vaccines is often impeded by a variety of obstacles, including but not limited to suboptimal efficacy in vaccine transfer, restricted capacity to elicit immune responses, and apprehensions regarding the safety of the constituent materials. Despite attempts to augment the immunogenic potential of vaccines, the development of a secure and noninvasive technique for delivering DNA vaccines persists as a multifaceted challenge. Duong et al. have developed an MN patch that exhibits pH responsiveness to augment DNA vaccination efficacy in human skin. The MNs are comprised of a polyelectrolyte multilayer composed of a charge-reversal, pH-sensitive copolymer and heparin. Moreover, DNA polyplexes are incorporated within the MNs via electrostatic interactions. The pH-responsive copolymer carries a positive charge at low pH but acquires a negative charge at physiological pH. Consequently, the MNs disintegrate, enabling the release of the DNA vaccine [137].

Choi and colleagues have devised MN that respond to insertion for the delivery of the canine influenza vaccine. The vaccine was lyophilized onto the tips of the MNs, which would separate from the base upon insertion and retraction. In both guinea pigs and hairy dogs, the vaccination stimulated antibody production that was equal to or exceeded that of the intramuscular injection. Furthermore, the immune response in guinea pigs resulted in a reduction in the duration of viral shedding to 8 days. Although these

insertion-responsive MNs do not meet the criteria of conventional responsive MNs due to their restricted performance in controlled delivery, they have effectively tackled the inconveniences of hair removal and could be considered as a viable alternative to conventional animal and human vaccinations [138,139]. He and colleagues have developed a microneedle (MNs) delivery system that employs synthetic pH-induced charge-invertible polymers (PDM) coated on the MNs to achieve strong immune responses. The MNs' surfaces possess a negative charge, with polyanions and polycations deposited on them. Upon introduction into the skin, the pH shifts to 7.4, causing the PDM layer to rapidly invert the charge, resulting in the consecutive layers repelling each other and detaching quickly. In vivo experiments conducted on mice demonstrated that the MNs could be administered rapidly within 1 min and had sustained release over 3 days [140]. Duong and colleagues have introduced a novel class of MN arrays that are ultra-pH-responsive and are designed for the purpose of delivering vaccines in the context of cancer immunotherapy. The MNs were coated using a layer-by-layer coating process with ultra-pH-responsive copolymers, DNA vaccines, and immunostimulatory adjuvant poly(I:C). In vivo studies were conducted on a mouse model that effectively demonstrated the induction of an immune response and the significant inhibition of melanoma cell growth [141].

Kim and colleagues have developed an MN system that employs chemoattractant *N*-formyl-methionyl-leucyl-phenylalanine peptide (fMLP)-releasing microspheres (fMLP-MS) to administer a polyplex-based DNA vaccine while simultaneously recruiting antigen-presenting cells (APCs). The MN is coated in a pH-responsive polyelectrolyte multilayer assembly (PMA) consisting of a polyelectrolyte layer-by-layer (heparin/albumin) construction created by immersing the MN alternately into heparin and albumin solutions, repeated eight times to produce 16 bilayers (Fig. 5). The assembly is terminated with a heparin layer as the outermost layer. Upon the application of the MN onto the skin, the polyelectrolyte multilayer assembly can be promptly disassembled. This disassembly occurs due to the pH shift to a physiological level upon MN application, which results in the release of polyplexes and fMLP-MS. The nearby cells, such as dermal epithelial cells and resident APCs, have the ability to uptake the released polyplexes, while the fMLP peptide is released from the MS to attract APCs through chemotactic effects. Moreover, the released polyplexes can effectively facilitate the cellular delivery of a DNA vaccine that encodes for a secretable Ab1-42 antigen. The MN-based vaccination system has demonstrated its capability in inducing robust antigen-specific antibody production [142].

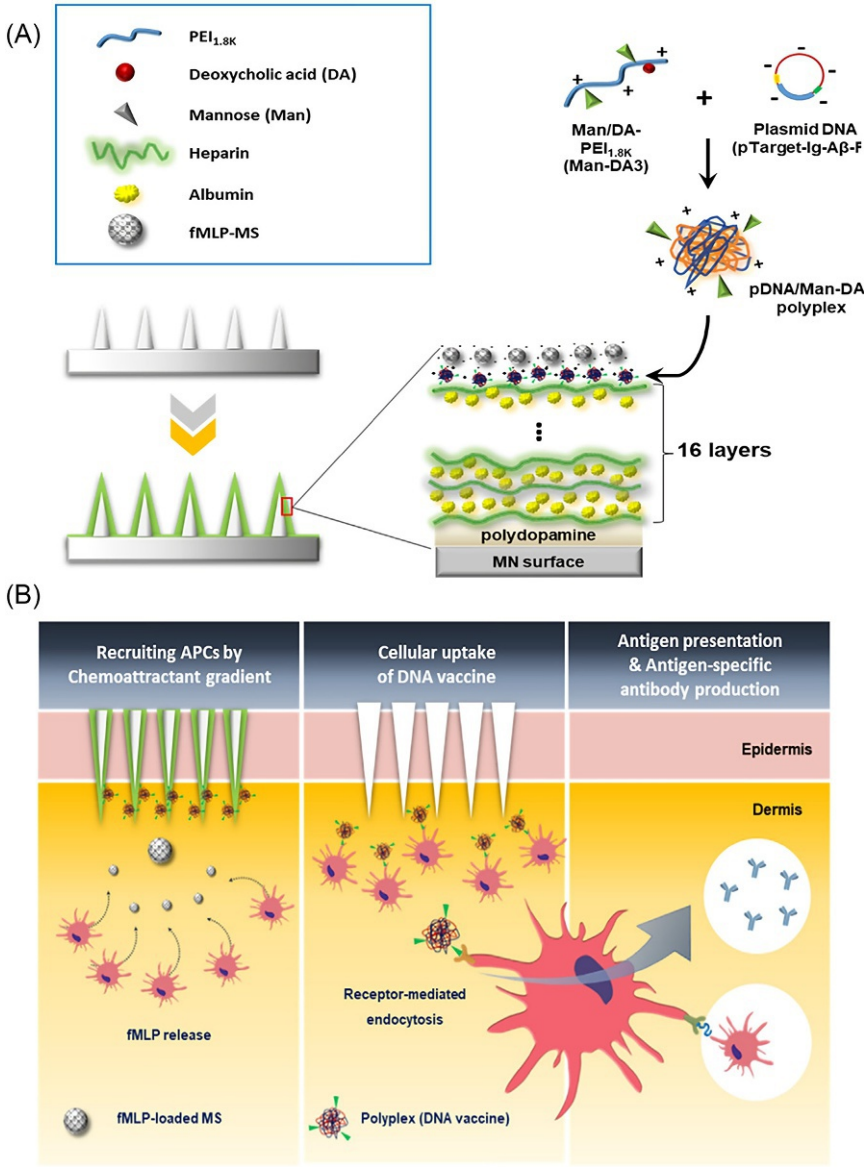

Fig. 5 (A) A schematic illustration of MN coated with pH-responsive PMA for the cutaneous delivery of polyplex containing a DNA vaccine and fMLP-MS. (B) The MN-mediated DNA vaccination process [142].

4.5 Heart diseases

Thrombosis, a medical condition marked by anomalous blood clotting, is a prevalent contributor to cardiovascular morbidity and mortality worldwide. The formation of intravascular blood clots may cause impediments in blood vessels, leading to organ impairment and severe cardiovascular complications. Anticoagulant medications, such as heparin, are often employed in cardiovascular patients to impede or delay the formation of such clots. The timely administration of these drugs is imperative for optimal disease management. Recently, a thrombin-responsive closed-loop patch has been developed to facilitate prolonged heparin delivery in a feedback-controlled manner. The present study details the utilization of microneedles in a novel patch designed for the detection of activated thrombin. Upon detection, the patch releases heparin to prevent blood clotting. The system is primarily comprised of a thrombin-cleavable peptide possessing a sequence of GGLVPR|GSGGC that is linked to the HA matrix containing heparin. By introducing this linker, the peptide can be cleaved specifically by thrombin, releasing heparin from the thrombin-responsive heparin-conjugated hyaluronic acid (TR-HAHP) matrix (Fig. 6A and B). Notably, the TR-HAHP matrix shows rapid release of heparin upon elevated concentrations of thrombin. As the levels of thrombin increased, the TR-HAHP matrix expeditiously released heparin, which exhibited the capability to hinder the formation of blood clots through the deactivation of thrombin. Furthermore, the thrombin-responsive microneedles exhibited efficacy in protecting mice from acute pulmonary thromboembolism for an extended

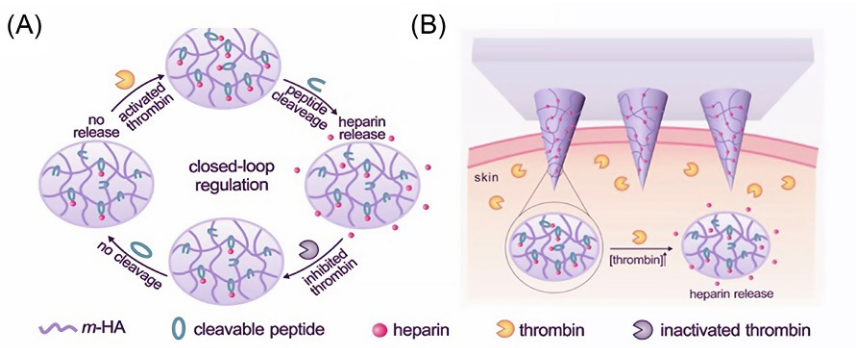

Fig. 6 (A) Formation and mechanism of the feedback-controlled heparin delivery system based on thrombin-responsive HAHP (TR-HAHP) conjugate. (B) Schematic of the TR-HAHP MN array patch in response to thrombin [143].

time period. In contrast, mice that were administered empty microneedles or microneedles that lacked the thrombin-sensitive peptide and did not respond to thrombin succumbed to thrombin injection within a mere 15-min interval [143].

Drug-eluting balloon (DEB) angioplasty is an effective treatment for cardiovascular and cerebrovascular diseases. However, the handling of DEBs can result in drug loss and rapid drug metabolization, leading to distal embolism and late lumen restenosis. To enhance the precision and efficacy of drug delivery through microneedles in blood vessels, Huang proposed the use of photothermal tip-separable microneedles drug-loaded balloons (MNDLBs). In the study, a solution containing drug-loaded tips (PCL, PLGA, and PTX) was prepared and cast onto the PDMS MN mold. The MN bases were made using a curable resin, and the MN array was transferred to the curved surface of the balloon. In contrast to conventional drug-eluting balloons, which exhibit weak bonding between the surface and drugs that are easily washed off, our specially-designed MN-tip drug-eluting balloon (MNDLB) encapsulates antiproliferative drug paclitaxel (PTX) within the MN tips. Upon insertion into the luminal lesion, the MN tips transmit the drug into the vessel wall. A ring-laser fiber is placed within the catheter's inner shaft and gradually removed with 808 nm near-infrared (NIR) light emission to generate heat and liquefy the phase-change material, lauric acid (LA), present in the MN tips. Upon cooling, the LA solidifies, thereby weakening the bond between the MN tip and the base. PTX is gradually released from the MN tips in the MNDLB group, with a 69.99 ± 21.46 μg/g drug concentration within the lesion after 180 days. In contrast, the DEB group exhibited a more rapid drug metabolism, with a 0.30 ± 0.16 μg/g drug concentration on day 30 and nearly 100% cumulative release [144].

4.6 Brain diseases

Spinal cord injuries (SCI) can lead to long-term disabilities that significantly affect patients' lives. Following a spinal cord injury, inflammation and cytotoxicity can cause additional damage to neural cells. This secondary injury's progression may be minimized by administering antiinflammatory drugs. By using microneedles, the drug can be released subdurally with minimal damage to the dura and minimal disturbance to the subdural space. Researchers developed a microneedle array coated with polypyrrole (PPy) using microscale 3D printing technology to enable localized delivery of dexamethasone, which targets the nervous system while minimizing dural

opening. This technology also allows for electronically controlled encapsulation and transdural release of drugs. In vitro and in vivo tests demonstrated that PPy microneedles can selectively release dexamethasone in a controlled manner. This system has the potential to reduce swelling in the spinal cord and traumatic brain injury. Applying electricity (1 V) for 2 min caused dexamethasone to be electrically secreted from the microneedles into wells containing cells, which resulted in reduced free radical production in an in vitro neuroinflammation model [145]. L-DOPA, a frequently utilized drug in the treatment of Parkinson's disease, is typically administered orally, although this method of delivery is associated with numerous digestive system discomforts and is prone to degradation by digestive juices and intestinal microorganisms, which results in low absorption efficiency and delayed efficacy of the drug. Zhou and colleagues have developed a unique microneedle patch that enables the controlled release of L-DOPA with both temporal and spatial precision, activated by near-infrared (NIR) light, for the treatment of Parkinson's disease. The microneedles' gelatin-methacrylate (GelMA) matrix is combined with therapeutic drugs, which are then stored in the mesopores of up-conversion micron-rods (UCMRs). The present study investigates the utilization of upconversion microrods (UCMRs) that contain Tm^{3+} and Yb^{3+} ions to convert near-infrared (NIR) light to ultraviolet (UV) and visible light. Upon exposure to 980 nm NIR irradiation, the UCMRs exhibit a photonic conversion of NIR to UV and visible light. The UV and visible light generated by the UCMRs in the microneedle system (UCMRs@mSiO2-azo-L-DOPA) can be assimilated by the azo molecules present within the system, which undergo photoisomerization from cis-azo to trans-azo. This leads to the controlled release of L-DOPA from the microneedle patch through the continuous oscillation of the azo molecules. The microneedles demonstrate a high degree of biocompatibility and do not exhibit any toxic effects toward RAW 246.7 cells. In addition, the concentrations of L-DOPA were observed to be high in both the blood and brain of a Parkinson's mice model [146].

4.7 Skeletal diseases

Skeletal muscles are crucial components of the human motion system. Moxibustion is a traditional Chinese medicine therapy that involves using infrared light generated by burning wormwood to locally heat damaged tissues and treat muscle conditions. However, the burning of wormwood during moxibustion treatment can increase the risk of local scalding and produce

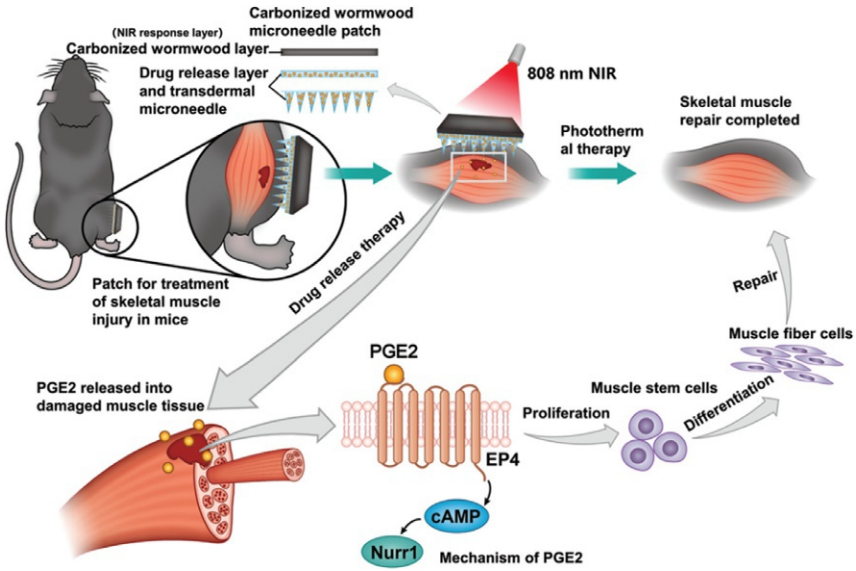

Fig. 7 Schematic diagram of the treatment of skeletal muscle injury with carbonized wormwood modified microneedle patches [147].

a significant amount of smoke that may lead to respiratory infections. Zhang and their team evaluated a smoke-free system based on moxibustion using smart microneedles to efficiently repair damaged skeletal muscles. In their study, they used polyvinylpyrrolidone (PVP) microneedles loaded with carbonized wormwood and prostaglandin G2 (PEG2). Similar to other NIR-responsive microneedles, carbonized wormwood heated the microneedles under infrared light to promote the release of PEG2. The in vivo experiments showed that the carbonized wormwood + PEG2 group exhibited superior induction of muscle stem cell proliferation and differentiation to repair damaged muscle tissue under heat therapy compared to the group without carbonized wormwood (Fig. 7) [147].

5 Conclusion

Microneedles that are sensitive to stimuli originating from the exterior environment have substantial potential for precise drug delivery in the treatment of various clinical conditions. However, these microneedles generally necessitate an auxiliary mechanism to initiate drug release from the applied patch. Conversely, microneedles that are responsive to stimuli originating from the

organism itself do not necessitate an external mechanism to regulate drug release, but their suitability for treating certain health conditions may be limited due to a dearth of appropriate internal stimuli. In these cases, microneedles that are manually or externally stimulated and responsive to exogenous stimuli play an imperative role in drug delivery. Electric current-responsive microneedles are among the most advantageous of exogenous stimuli-responsive microneedles due to their precise drug delivery ability and reduced complexity. Meanwhile, mechanical force-responsive microneedles are also admirable because they do not require additional equipment to trigger drug release. The rate of drug release from mechanical force-responsive microneedles can be precisely controlled through self-achievable stretching, bending, pressing, or insertion, without concern about the drug's structure. Therefore, this microneedle system could be an efficient, simple, and inexpensive tool for on-demand drug delivery. However, several comprehensive issues should be considered and fully optimized for clinical therapeutic applications, especially for light-activated microneedles. However, there are several issues that need to be addressed for optimal clinical outcomes when using light-activated microneedles. The exposure time and transition temperature should be precisely controlled to avoid potential thermal-induced skin injury or denaturation of chemotherapy drugs and other biomolecules. Furthermore, the use of nonbiodegradable and poorly biocompatible photothermal agents to generate heat from NIR irradiation can cause serious problems. In conclusion, using microneedles for drug delivery has great promise, but several factors, including biocompatibility and control of exposure time and temperature, must be carefully considered for optimal clinical outcomes.

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CHAPTER 5

Silicon microneedles for drug delivery

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1 Introduction

Miniaturized or microscale devices have gained much interest, mainly in the automotive, biomedical, integrated circuit, and industrial sectors [1]. In general, electronic devices are fabricated according to processing sequences of integrated circuits, which are bipolar metal-oxide-semiconductor processes or metal-oxide-semiconductor processes [2,3]. The devices usually are integrated with complementary metal-oxide semiconductor (CMOS) circuitry on a single chip to enable miniaturization and performance improvement. CMOS, also known as chips or microchips, is the most popular technology for assembling integrated circuit (IC) chips, such as digital logic circuits, microcontrollers, and microprocessors [4,5]. Microelectromechanical systems (MEMS) technology was introduced to produce miniaturized devices [6]. The integration of CMOS circuitry and MEMS technology has revolutionized the development of electronic devices, delivering compact and high-performance solutions that address the increasing demand for miniaturization across diverse sectors.

MEMS technology amalgamates mechanical aspects with sensing and physical movement in a single device [7]. Miniaturized devices, typically between a millimeter to micrometer in size, are created using semiconductor manufacturing processes, such as photolithography, wet and dry etching, and thin-film deposition. These processes are used to obtain the required shapes and functions [2,8]. MEMS devices have several prominent advantages, which include ease of integration with other devices, compact and lightweight structure, low power consumption, and increased sensitivity and resonance frequency [9–11].

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Biology and medicine apply MEMS technology, leading a new field, namely, BioMEMS or bio-microelectromechanical systems [2]. Researchers have been focusing on this important and exciting field of BioMEMS to create MEMS devices designed specifically for biomedical applications. In particular, there has been significant interest in microneedles that can be used for transdermal drug delivery and gene delivery [12]. Hypodermic needles are commonly used prior to the introduction of microneedles for transdermal drug and gene delivery. However, hypodermic needles have significant drawbacks, such as insertion pain due to the large needle tip diameter [13]. Microneedles were developed as a solution to the significant limitation of hypodermic needles, conceptualized as a painless, safe, and self-administered transdermal drug delivery tool [12]. Microneedles with lengths up to 1500 μm, widths ranging from 50 to 250 μm, and tip thickness between 1 and 25 μm can penetrate the outermost layer of the dermal skin structure, known as the stratum corneum, and reduce pain induced to the patient [14].

In general, microneedles can be grouped into five distinct types based on their delivery strategies. These include solid microneedles, hollow microneedles, coated microneedles, hydrogel-forming microneedles, and dissolving microneedles, as shown in Fig. 1 [15]. Several materials, such as polymers, ceramics, and metals, can be used for microneedle fabrication. However, silicon is the most widely used material owing to its high flexibility, which allows customizable manufacturing of microneedles with desirable shapes and sizes. Since silicon is only well suited to manufacture solid, hollow, and coated microneedles, this chapter will focus on these types. The different silicon microneedles have unique delivery strategies considering their distinct application, which we will delve into a detailed discussion in the following sections.

2 Types of silicon microneedles

Parallel with the advancement of BioMEMS in the 1990s, which utilized silicon both as a predominant structural material for MEMS devices and a substrate for semiconductor manufacturing equipment, it is not surprising that silicon is the earliest material used for microneedle fabrication [16,17]. Silicon is a well-known anisotropic crystalline material in which the orientation of the crystal lattice alignment influences its material behavior. Young's modulus of silicon ranges from 50 to 180 GPa [18], making it a versatile material due to its flexible nature that allows possible tailoring

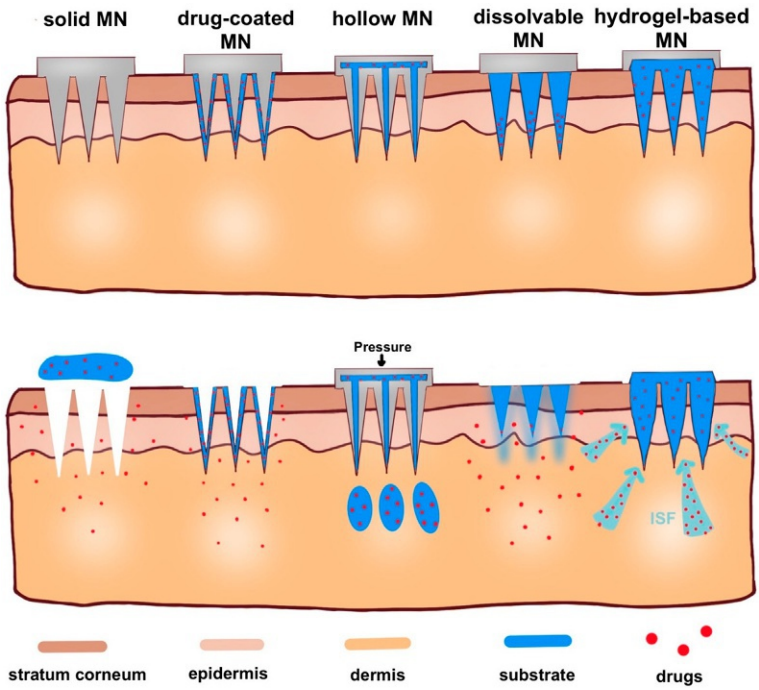


Fig. 1 Schematic representation of five types of microneedles: Solid, drug-coated, hollow, dissolvable, and hydrogel-based microneedles. *(Reproduced from J. Xu, D. Xu, X. Xuan, H. He, Advances of microneedles in biomedical applications, Molecules 26 (19) (2021), licensed under MDPI/Public Domain Creative Commons Attribution (CC BY) license 4.0. http://creativecommons.org/licenses/by/4.0.)*

to specific shapes and sizes of the microneedles. The highest mechanical strength of silicon (180 GPa) is comparable to that of SUS316L stainless steel [19], used in medical devices and implants, demonstrating that silicon has a desirable mechanical property. This has enabled silicon microneedles to successfully penetrate through human or mouse skin for the transdermal delivery of active molecules or drugs, such as insulin [20,21], galantamine (GAL) [22], meso-tetra (*N*-methyl-4-pyridyl) porphine tetra tosylate (TMP) [23], cholesterol-modified housekeeping gene (Gapdh) siRNA [24], and vaccine [25]. Although there is some concern about the biocompatibility of silicon, silicon has passed the baseline battery of ISO 10993 physicochemical and biocompatibility tests required by the Food and Drug Administration and other international regulatory agencies [26]. However, extra biocompatibility testing will be necessary to fulfill the ISO 10993 implant criteria [26].

2.1 Solid microneedles

As the name implies, solid microneedles take up solid structures, wherein structurally, they are much more robust and have stronger mechanical strength. The tips are more accessible to manufacture in comparison with hollow microneedles [27]. Solid microneedles are primarily used for skin pretreatment, which creates microchannels upon insertion into the skin and thus increases drug permeation following the topical application of drugs. The drug delivery method via solid microneedles employs the "poke-and-patch" approach, involving a two-step process [28]. The first step involves the penetration of a single or an array of solid microneedles through the stratum corneum barrier. This results in the creation of transient microchannels in the skin. The second step involves the application of a drug in the form of a patch, cream, solution, or gel to the area of the skin that has been penetrated following the removal of microneedles. This step allows the drugs to be passively diffused via a transdermal route. In vitro transdermal transport studies showed that the application of solid silicon microneedles could increase the skin permeability to macromolecules and particles by several orders of magnitude (up to 50 nm in radius) [29]. A high efficiency of intracellular delivery of the fluorescent dye calcein marker into viable cells and a high percentage of cell viability was also observed upon micrometer-scale piercing of cells using the solid silicon microneedles [29].

2.2 Coated microneedles

Coated microneedles also have a solid-core microneedle structure but with the addition of drug coating in the form of solid films on the microneedle surface. Following penetration into the skin, the coated microneedles could be withdrawn once the coating hydrates and dissolves. The action releases the drug from the surface into the skin. Hence, the drug delivery method for coated microneedles is commonly known as the "coat and poke" approach. Although the administration route is much simpler for coated microneedles as opposed to the two-step process for solid microneedles, only a small volume of drugs can be delivered via coated microneedles since the surface area of the microneedles is small, which reduces its delivery efficiency [30]. Consequently, multiple coating processes will be required to achieve a sufficient dose that may ultimately blunt the microneedles. Thus, coated microneedles are said to be more suited for delivering very potent drugs or vaccines. In the case of vaccines, only small quantities of antigens are needed to produce an immune response [31,32]. An approach to overcome this limitation is by

using methods to obtain nanometer-thin drug coatings applied on microneedles, such as assembling layers one at a time to create polyelectrolyte multi-nanolayers combined with pH-sensitive surface modification [33]. Van der Maaden et al. [33] utilized this layer-by-layer assembly method by producing 10 layers of inactivated polio vaccine (IPV) alternated with an *N*-trimethyl chitosan chloride (TMC). The approach took advantage of the binding between the positively charged microneedle surface that was chemically modified with pH-sensitive pyridine groups and the negatively charged antigen IPV to bind IPV onto the microneedle surface [33]. The authors showed that the IPV and TMC were successfully released into the conduits that were formed following the application of IPV-TMC-coated pH-sensitive microneedles onto ex vivo human skin, demonstrating that the coating only slightly reduced the sharpness of microneedles [33]. Several other coating techniques that are currently available include gas jet dry coating [34], dip coating, e.g., masked dip coating [35], thin-film dip coating [36], and coating by immersion [37].

2.3 Hollow microneedles

Hollow silicon microneedles employ the poke-and-flow approach, similar to the traditional hypodermic needles, but much shorter in form. These hollow bore or side-opened microneedles form channels that allow efficient delivery of drugs either through diffusion, pressure-driven, or electrically driven flow. This advantageously allows more significant quantities of drug substances to be delivered than the other microneedles [38]. However, they are more susceptible to becoming clogged by microscopic debris during penetration into the skin and possess poor mechanical strength due to their structure [21]. The advantages and disadvantages of the different types of microneedles are exemplified in Table 1.

3 Manufacturing techniques for silicon microneedles

The earliest silicon microneedle made is initially referred to as micromechanical piercing structures with pyramidal shapes called "microprobes" [16,17]. These "microprobes" were fabricated from silicon by bulk micromachining and were purposefully used for genetically transforming tobacco cells and nematodes. With a height of around 80 μm, the sharp tips of "microprobes" permeated the cell walls of tobacco cells and nematodes, allowing genetic materials to pass through the cell walls [16,17]. Afterward, Henry et al. [39] fabricated solid silicon microneedles using the reactive ion

Table 1 The strengths and weaknesses of the three types of microneedles.

Microneedle types	Advantages	Disadvantages
Solid	**(1)** Possess desirable mechanical strength **(2)** Large molecules can be administered	**(1)** The two-step process involved may cause impractical issues for some patients **(2)** Poor dose accuracy **(3)** Potentially cause infection if reused
Coated	**(1)** Possess desirable mechanical strength **(2)** Involves a single-step process	**(1)** The quantities of drugs that can be supplied rely on the coating layer's thickness
Hollow	**(1)** Large doses of drugs can be administered **(2)** Accurate dose administration **(3)** Drug flow rate can be controlled by flow-control equipment	**(1)** Poor mechanical strength **(2)** It may cause clogging

etching process with a chromium mask and demonstrated the first transdermal delivery of drugs using solid silicon microneedles. They showed that the array of silicon microneedles with an approximate length of 150 μm was successfully inserted into the stratum corneum side of the human epidermis in vitro to facilitate the delivery of calcein (model drug) with 10 N force [39].

Silicon microneedles are mainly fabricated using MEMS methods. Processes requiring MEMS methods are relatively expensive and necessitate cleanroom facilities. However, other methods that include mold-based techniques, e.g., hot embossing [40], laser machining [41], and non-mold-based techniques, such as 3D printing [42,43], are unsuitable with silicon as the structural material for microneedle fabrication. In addition, these methods cannot offer submicrometer sharpness and produce somewhat lower-resolution microneedles. The microneedles may also be incompatible with microdevices [44,45]. Thus, the MEMS methods remain the main fabrication techniques for silicon microneedles fabrication, and these methods are still prevalent today. The fabrication processes of MEMS methods can be divided into three main steps: deposition, patterning, and etching [46,47].

- *Step 1—Deposition.* In the first step, deposition involves creating a thin substrate film with a thickness ranging from a few nanometers to about

100 μm. A broad range of methods can be used to deposit the thin film of material (e.g., silicon, silicon oxide, silicon nitride) onto a substrate (usually a silicon wafer with a thickness of 300–700 μm) that includes chemical or physical vapor deposition (CVD or PVD), physical deposition (spin coating, electron beam evaporation, or sputtering), and thermal oxidation [46,48]. The two most used CVD methods are low-pressure CVD (LPCVD), which produces a uniform and conformal coating of film at higher temperatures (over 600°C), and plasma-enhanced CVD (PECVD), which enables deposition at lower temperatures. Of those, LPCVD appeared to be the most frequently used method for thin-film deposition on the substrate for silicon microneedle fabrication [22,23,49,50]. Thermal oxidation using the wet oxidation process is also used to obtain the oxide layer [51–53].

- *Step 2—Patterning.* After the film substrate has been deposited, the patterning process is carried out by transferring a two-dimensional master design from the original photomask to the photosensitive film on the substrate, referred to as the lithography method [46]. Different lithographic methods could be used depending on the radiation exposure, including optical lithography (photolithography), electron beam, x-ray , and ion beam lithography. Of the different types of lithographic methods, photolithography is the most commonly used to fabricate silicon microneedles. Photolithography uses UV light ($\lambda = 193$–236 nm) that passes through a defined pattern, creating the desired pattern on a substrate.
- *Step 3—Etching.* The last fabrication step involves an etching process of the patterned wafer where the uncovered silicon area is vertically etched, and a slight lateral under-etched region occurs to produce an array of pointed silicon microneedles [54]. The etching process can either be a dry or wet etching or a combination of both methods. Most silicon microneedles were fabricated using deep-reactive ion etching (DRIE), a dry etching technique also referred to as the Bosch process [55]. This technique is performed at low pressure in the presence of inert or reactive gases that do not involve liquids. DRIE commonly uses silicon etchant and sulfur hexafluoride ($SF_6 + O_2$) as etchant. The switching of gas feed between $SF_6 + O_2$ (etch) and C_4F_8 (sidewall passivation) in the Bosch process results in the production of a high aspect ratio (ratio of needle's height to the base width) of silicon dry etching [56]. Besides dry etching, wet etching is also involved in constructing silicon microneedles. The wet etching technique involves the removal of unwanted materials by immersing the wafer in a liquid bath containing a chemical etchant.

Isotropic and anisotropic etching are the two primary wet etching methods. The application of isotropic etchants, e.g., nitric acid or hydrofluoric acid, will lead to isotropic etching, which will remove the materials from a substrate under the etch mask. The use of anisotropic etchants, for instance, tetramethylammonium hydroxide (TMAH), potassium hydroxide (KOH), ethylenediamine (EDP), and hydrazine, will result in the etching of the material (silicon wafer) at different rates and directions, generating more controlled shapes of microneedles [57].

Over the two decades after the first fabrication of silicon microneedles [16], a wide array of silicon microneedles have been fabricated differing in designs, lengths, shapes (e.g., canonical, tapered tip, pyramidal, cylindrical), and densities. Fig. 2 shows two designs available based on the orientation of microneedles against the substrate: (a) in-plane microneedles, which can be defined by microneedles having an orientation parallel to the fabrication surface or base substrate, and (b) out-of-plane microneedles possessing an orientation perpendicular or 90 degrees to the fabrication surface. The production of in-plane microneedles allows more flexibility in shapes, sizes, and applications. They can be produced in a wide range of lengths and integrated with other components or devices, such as microfluidic devices [58,59]. Some in-plane microneedles are also CMOS-compatible [60]. However, several significant drawbacks of in-plane microneedles are that they can only be produced in low density because of the one-dimensional (1-D) arrays of fabrication. Due to the control of film or doped layer

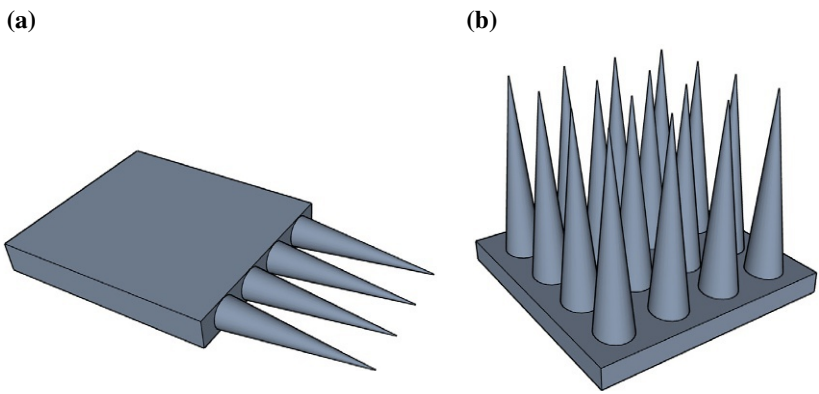


Fig. 2 Two distinct designs based on the orientation of microneedles against the substrate: (A) in-plane microneedles having an orientation parallel to the fabrication surface, and (B) out-of-plane microneedles with an orientation perpendicular or 90 degrees to the fabrication surface.

thickness, increasing lumen and lateral shaft dimensions beyond a few or tens of micrometers is unfeasible, making them fragile [61]. On the other hand, out-of-plane microneedles could be produced with a higher density of arrays, which could increase the efficacy of transdermal delivery of drugs. Out-of-plane microneedles' lumen and lateral shaft dimensions could be controlled lithographically, making them more robust than in-plane microneedles [61]. Nevertheless, creating out-of-plane microneedles with a high aspect ratio can be challenging, and integrating them with microfluid chip systems and other microdevices can be difficult.

3.1 Out-of-plane silicon microneedles

As the stratum corneum and live epidermis thicknesses are 10–40 μm and 50–100 μm, respectively, the microneedles should have a length of at least 150 μm to ensure that the drugs or active molecules reach the deeper tissue layer. The first attempt at producing solid out-of-plane silicon microneedles for transdermal delivery of drugs successfully produced 150-μm-long canonical-shaped microneedles. They used a single-step DRIE technique for fabrication [39]. The patterned silicon wafer from the lithography process was subjected to reactive ion etching using the silicon etchant $SF_6 + O_2$ [39]. Although the strength of the microneedles needed to be improved since insertion into human skin resulted in a few needles bent [39], the study provided a good foundation for later studies on the fabrication process of silicon microneedles using the MEMS processing steps.

Most manufacturing procedures for solid out-of-plane silicon microneedles utilized the combination of dry and wet etching techniques [22,23,49,50,53,62–65], while only several studies solely utilized dry etching [66]. Pradeep Narayanan et al. [62] fabricated a high aspect ratio (1.43) of tapered-shaped solid silicon microneedles using a single-step lithography and anisotropic TMAH wet etching. Optimization in the concentration of TMAH produced a higher vertical etching rate than the lateral etching rate, resulting in microneedles with a high aspect ratio [62]. Roh et al. [66] generated an even higher aspect ratio of microneedles of up to 1:25, where the microneedles have convex/irregular profiles of tips with various heights in a given array (Fig. 3A). They introduced simple fabrication methods employing one single photolithography and two subsequent deep-reactive ion etching (DRIE) processes to produce these high-density microneedle arrays of up to 625 microneedles mm^{-2}. Interestingly, Li et al. [22] fabricated super-short solid out-of-plane silicon microneedles with a

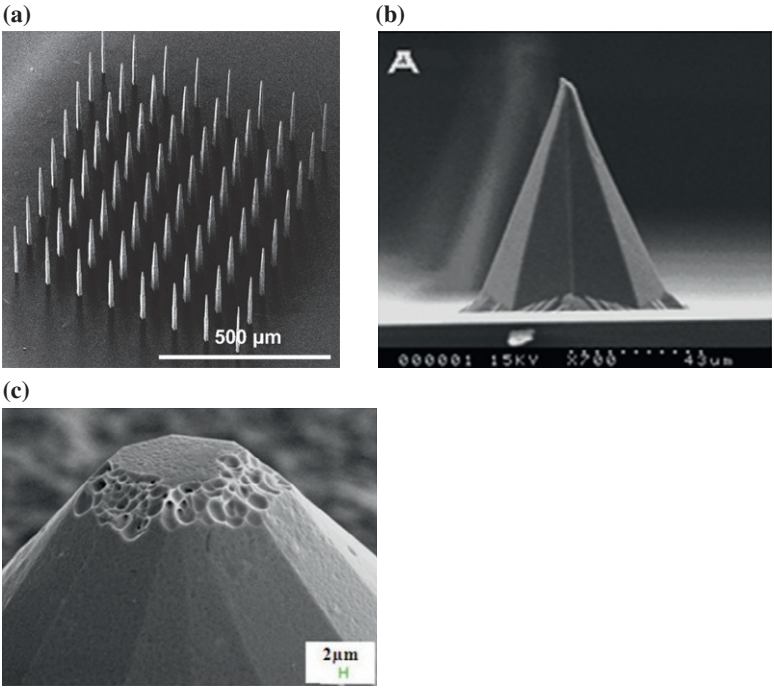


Fig. 3 Out-of-plane SEM images of solid silicon microneedles from (A) Roh et al. [66], (B) Li et al. [22], (C) Das et al. [67]. *(Panel A: Reproduced from H. Roh, Y.J. Yoon, J.S. Park, D.H. Kang, S.M. Kwak, B.C. Lee, et al., Fabrication of high-density out-of-plane microneedle arrays with various heights and diverse cross-sectional shapes, Nanomicro Lett. 14 (1) (2021) 24, licensed under Creative Commons Attribution (CC BY) license 4.0. http://creativecommons.org/licenses/by/4.0; Panel B: Reprinted from W.Z. Li, M.R. Huo, J.P. Zhou, Y.Q. Zhou, B.H. Hao, T. Liu, et al., Super-short solid silicon microneedles for transdermal drug delivery applications,* Int. J. Pharm. *389 (1–2) (2010) 122–129. Copyright (2010), with permission from Elsevier; Panel C: Reprinted from A. Das, C. Singha, A. Bhattacharyya, Development of silicon microneedle arrays with spontaneously generated micro-cavity ring for transdermal drug delivery,* Microelectron. Eng. *210 (2019) 14–18. Copyright (2019), with permission from Elsevier.)*

70–80 μm length via dry and KOH wet etching, as shown in Fig. 3B. The authors demonstrated that the flat-tipped super-short microneedles array was more effective compared to sharp-tipped super-short microneedles when pierced into human skin. The reason was that the flat-tipped created better conduits to effectively facilitate the galantamine hydrophilic compound (GAL) transport across the rat skin [22]. Fig. 3C shows that in a recent study by Das et al. [67], pyramidal silicon microneedle arrays with microcavities with size 4–10 μm in diameter were fabricated using a combination of

crystallographic etching and thermal oxidation, followed by HNA treatment. Microcavities generated at the tip of the microneedle due to variations in the oxidation and etch rates can be used for transdermal drug delivery with a total volume of up to 600 nL [67]. Another interesting paper by Hamzah et al. [50] showed that concave conic-shaped solid silicon microneedles with a high aspect ratio were fabricated using hydrofluoric acid–nitric acid–acetic acid (HNA) etching. The etching process was optimized by varying factors, including the HNA composition, the optical mask's window size, etching temperature, and bath agitation [50]. The researchers found that the HNA etching parameter combination with an HNA composition of 2:7:1 (HF: HNO_3: CH_3COOH) with a window size of 500 μm and an agitation rate of 450 RPM produced the highest aspect ratio (1.44) microneedles compared to other parameter combinations (Fig. 4) [50].

To our knowledge, very little literature has been reported on the coated-type out-of-plane silicon microneedles. Griffin et al. [25] manufactured coated-type silicon microneedles named Nanopatch, consisting of an array of 10,000 silicon microprojections per cm^2, which can be dry-coated with a vaccine. The microprojections exhibited 250 mm in length and a 40 mm base width with a tapered shape. They were fabricated via photolithography, etched with deep-reactive ion etching, and subsequently attached to the applicator [25]. Fernando et al. [68] also utilized Nanopatch but coated the device with a vaccine using the gas jet coating technique [34]. In another study, McGrath et al. [69] fabricated 280-μm out-of-plane silicon microneedles using wet etch technology as Wilke et al. [70]. They successfully used a conventional film-coating process to coat an intact film of biocompatible polymers (hydroxypropylmethylcellulose (HPMC) and carboxymethylcellulose (CMC)) on the microneedle arrays by optimizing spray coating parameters, such as concentration of coating material, liquid input rate, and atomization air pressure [69].

Like solid-type microneedles, fabrication of the opened-bore form of hollow, out-of-plane microneedles employed either the dry/plasma-based etching [20,52,71–76] or a combination of both dry and wet etching [21,44,77–80]. In the latter case, the wet etch process creates the overall needle structure, while the dry etch process, commonly DRIE, creates the inner bore on the backside or rear side of the substrate, in which the two processes can be performed interchangeably. Using the combination of both types of etching, O'Mahony et al. [44] developed a much simpler method for fabricating hollow silicon microneedles. It involves only two etch steps—a wet etch step using KOH solution followed by a dry etching process.

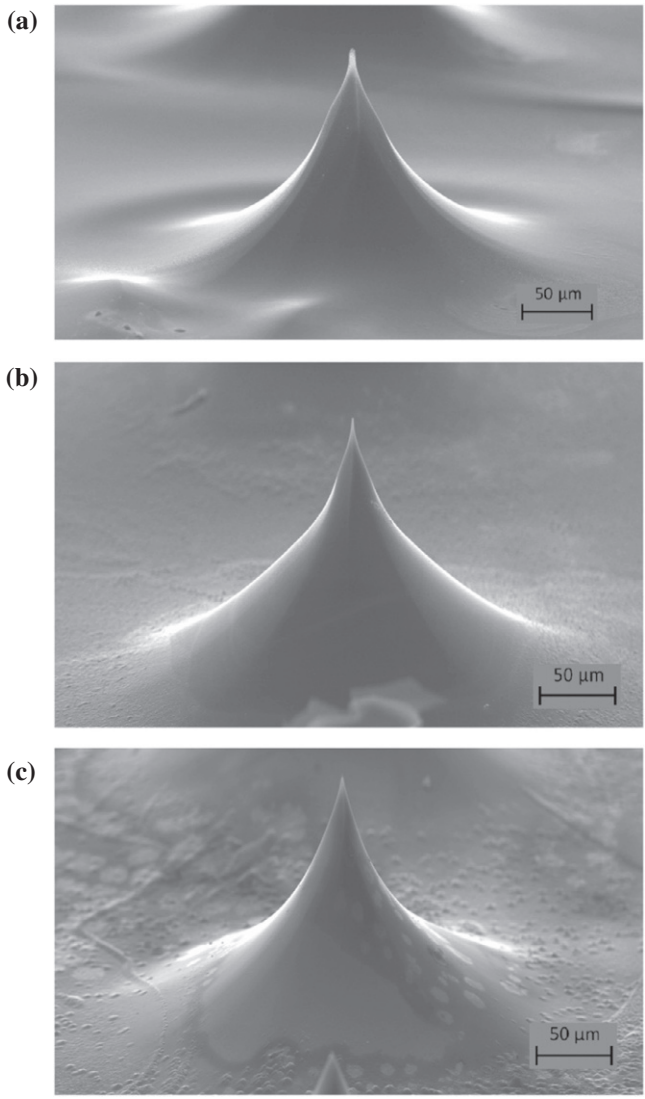


Fig. 4 SEM images of concave conic-shaped solid microneedles produced by (A) 400 RPM agitation, 500-µm window, 2:7:1 HNA composition (aspect ratio: 1.35), (B) 450 RPM agitation, 400-µm window, 3:6:1 HNA composition (aspect ratio: 1.33), and (C) 450 RPM agitation, 500-µm window, 2:7:1 HNA composition (aspect ratio: 1.44). *(Reproduced from A.A. Hamzah, N. Abd Aziz, B. Yeop Majlis, J. Yunas, C.F. Dee, B. Bais, Optimization of HNA etching parameters to produce high aspect ratio solid silicon microneedles,* J. Micromech. Microeng. *22 (2012) 095017. Copyright (2012), with permission from IOP Publishing.)*

Fig. 5A shows that the needles of up to 500-μm height and tip radii below 100 nm were fabricated. The fewer bulk etch processes than the three or four etches typically employed [21,77] could reduce manufacturing costs, complexity, and processing time [44]. Likewise, in a patent-pending three-step process utilizing only the dry etching technique, Bolton et al. [74] manufactured multiple arrays of sharp-tipped, hollow silicon microneedles (Fig. 5B),

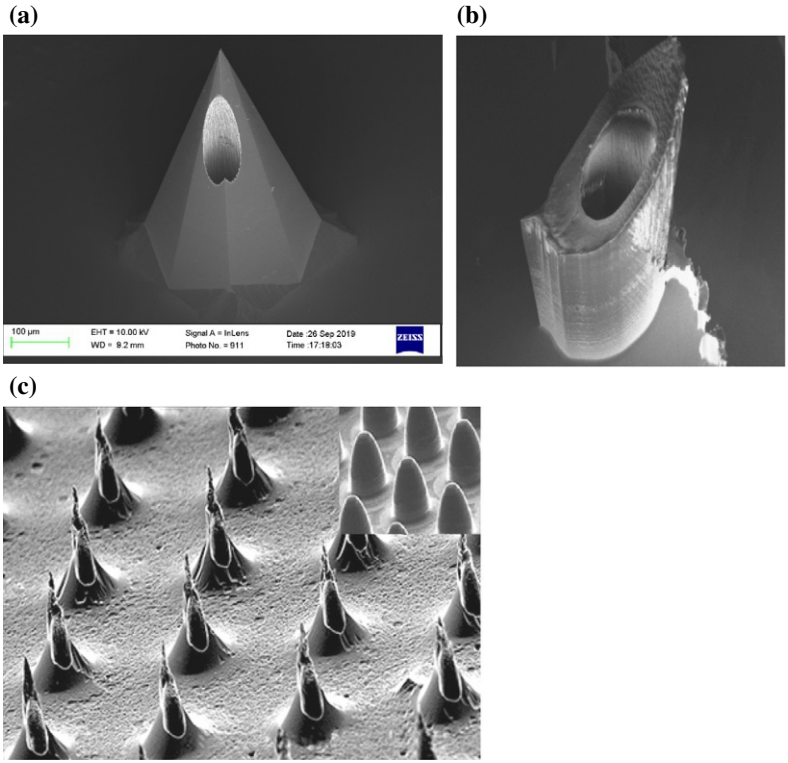


Fig. 5 Out-of-plane SEM images of hollow silicon microneedles from (A) O'Mahony et al. [44], (B) Bolton et al. [74], (C) Li et al. [80]. *(*Panel A: From C. O'Mahony, R. Sebastian, F. Tjulkins, D. Whelan, A. Bocchino, Y. Hu, et al., Hollow silicon microneedles, fabricated using combined wet and dry etching techniques, for transdermal delivery and diagnostics, Int. J. Pharm. 637 (2023) 122888*;* Panel B: From C.J.W. Bolton, O. Howells, G.J. Blayney, P.F. Eng, J.C. Birchall, B. Gualeni, et al., Hollow silicon microneedle fabrication using advanced plasma etch technologies for applications in transdermal drug delivery, Lab Chip 20 (15) (2020) 2788–2795; Panel C: From Y. Li, H. Zhang, R. Yang, Y. Laffitte, U. Schmill, W. Hu, et al., Fabrication of sharp silicon hollow microneedles by deep-reactive ion etching towards minimally invasive diagnostics, Microsyst. Nanoeng. 5 (1) (2019) 41, licensed under Creative Commons Attribution (CC BY) license 4.0. *http://creativecommons.org/licenses/by/4.0.)*

thus benefiting from the fewer steps involved and avoiding hazardous wet chemicals. Fig. 5C shows a modified triple-phase DRIE (i.e., passivation, depassivation, etching) was developed by Li et al. [80] to create microneedles with sufficiently deep holes (depth of >300 μm) with small diameters (as small as 30 μm) from a single side of the Si wafer. The resultant microneedles with the off-center location of the borehole from the needle axis are similar to side-opened hollow silicon microneedles produced by Roxhed et al. [71]. This microneedle design can minimize the risk of clogging at needle openings. However, the fabrication process in Roxhed et al. [71] involved consecutive multifaceted processes alternating between isotropic (unbiased) SF_6 plasma etching and anisotropic DRIE.

3.2 In-plane silicon microneedles

The fabrication of in-plane microneedles poses restrictions on the microneedle width (depending on the substrate diameter) and sharpness and produces a low-density microneedle array [58,59,81]. For example, in a study by Paik et al. [58], only five in-plane microneedles were produced. However, the device can be integrated advantageously with microfluidic systems (Fig. 6). Unfortunately, only a few studies have been conducted

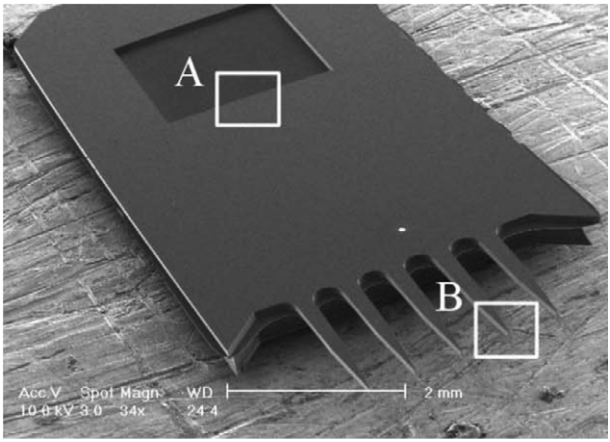


Fig. 6 In-plane silicon microneedle. *(Reproduced from S.-J. Paik, S. Byun, J.-M. Lim, Y. Park, A. Lee, S. Chung, et al., In-plane single-crystal-silicon microneedles for minimally invasive microfluid systems,* Sensors Actuators A Phys. *114 (2) (2004) 276–284. Copyright (2004), with permission from Elsevier.)*

on this type of microneedle. A recent study by Howells et al. [82] demonstrated the fabrication of solid and hollow in-plane silicon microneedle arrays of varied lengths from a single wet etch process using KOH. Utilizing the CVD process, 2.5 μm of silicon dioxide was deposited on both sides of a silicon wafer (300 μm thick with a (100) orientation). This was followed by photolithography to pattern devices onto both sides of silicon dioxide. The SPTS Technologies Inductively Coupled Plasma (ICP) system was used to etch a silicon dioxide layer window, which exposed the silicon substrate. Simultaneous front and back side etching of a (100) silicon wafer in 44% KOH solution was subsequently carried out to obtain the characteristic 54.7 degrees sidewall etch angle of V-shaped grooves, forming a sharp pyramidal six-sided microneedle solid silicon microneedle (Fig. 7). To create hollow microneedles, bonding two grooved microneedles together created an enclosed hollow channel [82]. The channel depth that can be etched depends on the width of the channel mask. A roughly 231-μm-wide and 67-μm-deep channel was created using a 200 μm channel mask width (Fig. 8A and B). Because the 54.7 degrees (111) sidewalls have not been etched deeply enough to connect and form the distinctive V-shaped channel, the channel's base is flat. On the other hand, the 100 μm channel mask width created a groove that was 134 μm wide and 88 μm deep, and the intersection of the (111) side walls successfully created a V-shaped channel (Fig. 8C and D) [82].

Jung et al. [83] also fabricated in-plane silicon microneedles of six types of needle tips with various shapes and tapered angles (isosceles triangles: 5 degrees, 10 degrees, and 30 degrees, right triangles: 10 degrees, 70.5 degrees, and 90 degrees). The microneedles were integrated with microfluidic channels inside the microneedles. They fabricated the microneedles using photolithography, crystalline wet etching processes on (110) silicon, and KOH solution. Nonetheless, compared to Howells et al. [82], the processes involved a multistep KOH wet etch process. In a recent study, Mamun et al. [60] fabricated in-plane silicon microneedles by post-CMOS-compatible microfabrication processes. They showed that after tip sharpening via etching, the maximum bending stress and the insertion, free bending, and maximum buckling forces were decreased, suggesting that their microneedles are robust and can penetrate the skin without much pain [60].

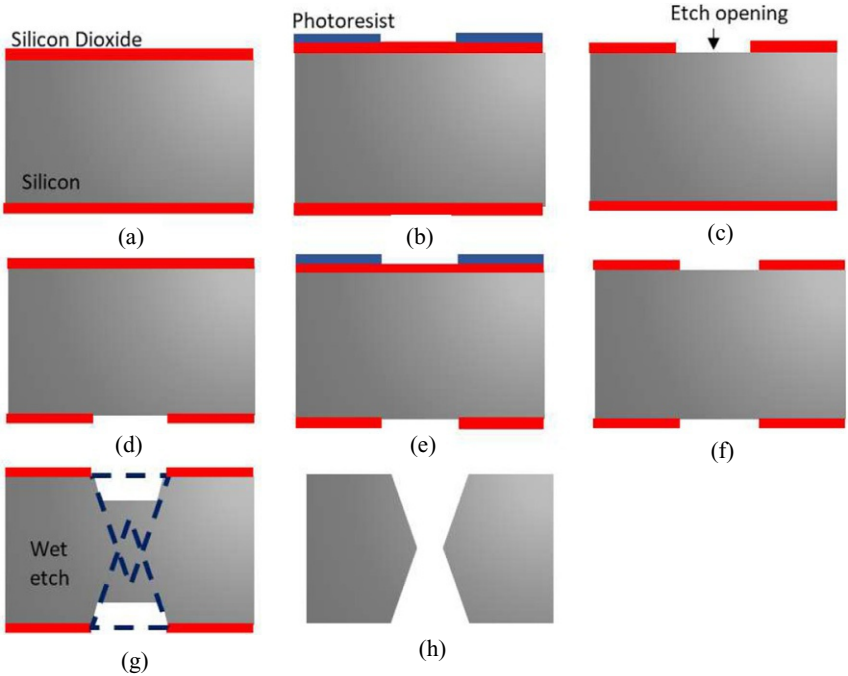


Fig. 7 Schematic representation of the cross-section of stages involved in fabricating in-plane silicon microneedle. *Gray* represents silicon wafers, *red* represents silicon dioxide, and *blue* represents photoresist. (A) 300-μm wafer is deposited with 2.5 μm of silicon dioxide on both sides using chemical vapor deposition (CVD). (B) Photolithography is used to transfer the pattern of the mask onto the silicon dioxide. (C) ICP is used to etch the device pattern into the silicon dioxide hard mask. (D) The wafer is subsequently flipped over. (E) Devices are patterned onto the reverse using photolithography, guided by the alignment markings. (F) ICP is used to etch the device pattern onto the silicon dioxide hard mark. (G) Simultaneous etching of the front and back sides of the wafer using 44% KOH solution is depicted by the *dashed lines*. (H) Removal of KOH solution from the devices. *(Reproduced from O. Howells, G.J. Blayney, B. Gualeni, J.C. Birchall, P.F. Eng, H. Ashraf, et al., Design, fabrication, and characterisation of a silicon microneedle array for transdermal therapeutic delivery using a single step wet etch process,* Eur. J. Pharm. Biopharm. *171 (2022) 19–28. Copyright (2022), with permission from Elsevier.)*

4 Clinical trials

4.1 Animals

Microneedles are a promising technology, but their effectiveness needs to be thoroughly tested before they can be used in medical applications. Researchers commonly use mice, rabbits, guinea pigs, and monkeys to study

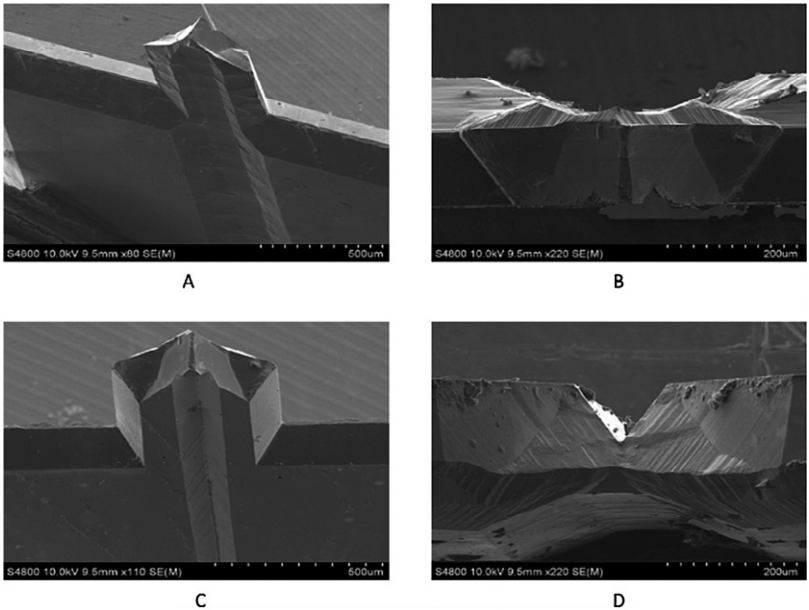


Fig. 8 Grooved microneedles with channel SEM images with two different starting mask widths—(A) and (B) are 200 μm while (C) and (D) are 100 μm. (B) and (D) show the orientation below the channel to examine the KOH etched shape. *(Reproduced from O. Howells, G.J. Blayney, B. Gualeni, J.C. Birchall, P.F. Eng, H. Ashraf, et al., Design, fabrication, and characterisation of a silicon microneedle array for transdermal therapeutic delivery using a single step wet etch process,* Eur. J. Pharm. Biopharm. *171 (2022) 19–28. Copyright (2022), with permission from Elsevier.)*

their performance to achieve this. Using these animal models, we can gain valuable insights into the safety, toxicity, tested compound, and efficacy of microneedles, which will ultimately help develop better treatments for various medical conditions [84,85].

Mechanical properties such as axial force, transverse force, insertion force, mechanical stability, and bending breakage force must be tested on different skin types to determine the failure force of microneedles. The aforementioned animals are used to characterize the mechanical properties as they can mimic human skin's subcutaneous tissues [12,86]. The insertion of microneedles into the skin is resisted by a resistive force in all skin types. Thus, the applied load on the tip should be higher than the skin's resistive force of approximately 0.03 mN or $3.18 \times 10^6\,\mathrm{N\,m^{-2}}$ [39]. Zainal Abidin et al. [64] performed stress and buckling analyses of concave conic-shaped silicon microneedles [50] by loading various loads ranging from 50 to

800 g perpendiculars to the tip of the microneedles array. Silicon microneedles were applied and pressed to the trypan blue-dyed rat skin along with the axial loads (Fig. 9A and B). Results showed that no significant stress deformation was observed when a 50 g load was applied. A 3D stimulation plot of the stress surface at the applied load of 50 g showed that the stress position was observed at the tip of the microneedle (Fig. 9C). The region in red color, as shown in the inset of Fig. 9C, indicated that the maximum stress

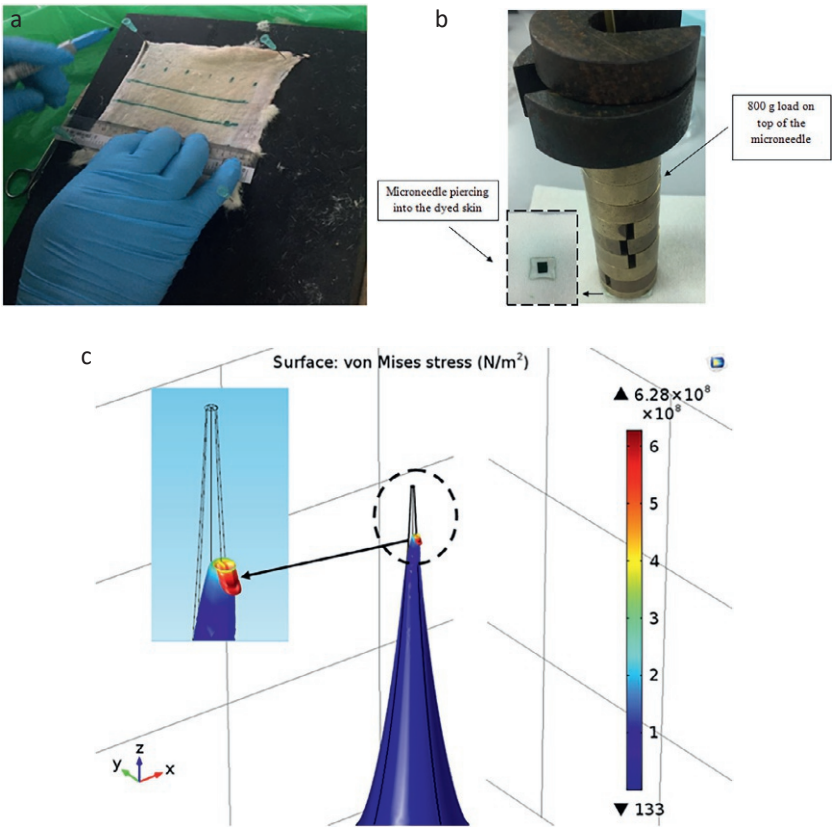


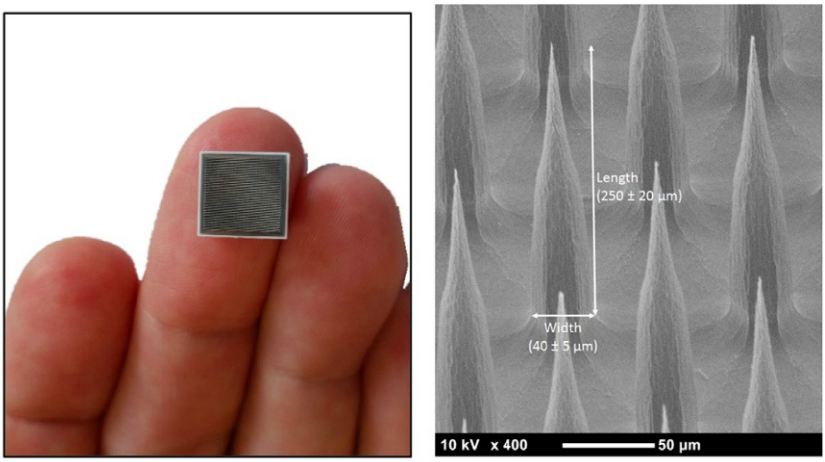
Fig. 9 (A) Rat skin cut into 2 cm × 2 cm smaller pieces. (B) 800 g load on top of microneedle and dyed skin. The inset shows the microneedle piercing into the dyed skin. (C) von Mises stress analysis for the applied load of 50 g. The inset shows the magnified plot for the surface deformation at the tip of the microneedle. *(Reproduced from H.E. Zainal Abidin, P.C. Ooi, T.Y. Tiong, N. Marsi, A. Ismardi, M. Mohd Noor, et al., Stress and deformation of optimally shaped silicon microneedles for transdermal drug delivery,* J. Pharm. Sci. *109 (8) (2020) 2485–2492. Copyright (2020), with permission from Elsevier.)*

value was $6.28 \times 10^8\,\mathrm{N\,m^{-2}}$ (the maximum load the tip can support before fracturing). Even when the load was increased progressively up until 800 g, the same stress value of $6.28 \times 10^8\,\mathrm{N\,m^{-2}}$ was discovered, and no fracture was noticed. This suggested that the same stress value was reached during the simulation due to the von Mises stress operating on the microneedle being lesser than the silicon's yield strength of $7 \times 10^9\,\mathrm{N\,m^{-2}}$ [64]. In another study, Donnelly et al. [23] used excised murine and porcine skins to investigate the application of silicon microneedles as a skin pretreatment to enhance the intradermal delivery of a preformed photosensitizer. The hydrophilic photosensitizer used in the study was a bioadhesive patch containing meso-tetra (*N*-methyl-4-pyridyl) porphine tetra tosylate (TMP). This drug improves topical photodynamic therapy (PDT) of skin tumors by inducing cell death in tumor cells [87]. The pyramidal-shaped solid silicon microneedles were fabricated using wet etch technology as produced by Wilke et al. [49] and were arranged on the silicon chips as a 6×7 array. Donnelly et al. [23] successfully showed that TMP can be delivered intradermally in vitro and in vivo in animal skin models, paving the way for further promising studies on the application of microneedles in treating skin tumors.

4.2 Human

Traditional needle and syringe vaccinations may be disadvantageous, attributed to the risk of needle-stick injury, the need for trained personnel, and vaccine reconstitution. Griffin et al. [25] produced a microprojection area of 9×9 mm with a microprojection density of $10{,}000\,\mathrm{cm^{-2}}$ tapered-shaped silicon microneedles that can be dry-coated with a vaccine (Fig. 10) to overcome these challenges (see Section 3.1 for manufacturing techniques). Nanopatch was tested on 18 healthy adults aged 18–45. In this study, the tolerability and acceptability of Nanopatch were tested but without vaccine antigens. The researchers demonstrated that applying the product to human skin is safe and tolerable [25]. It is also reported that 83% of the subjects preferred Nanopatch over the traditional needle and syringe administration. This breakthrough marks the beginning of a new era in the field of vaccination, where Nanopatch can undoubtedly be considered a game-changer [25].

Fernando et al. [68] further tested the Nanopatch and conducted human randomized, placebo-controlled trials. However, this time, the Nanopatch was coated with a vaccine for the inactivated influenza virus (A/California/



A B

Fig. 10 Uncoated silicon microneedles and microprojections. (A) Photograph of microneedle (10 × 10 mm) on a white polycarbonate backing. (B) SEM image of microprojections, including specific measured dimensions. *(Reproduced from P. Griffin, S. Elliott, K. Krauer, C. Davies, S. Rachel Skinner, C.D. Anderson, et al., Safety, acceptability and tolerability of uncoated and excipient-coated high density silicon micro-projection array patches in human subjects,* Vaccine *35 (48 Pt B) (2017) 6676–6684, under Creative Commons Attribution (CC BY) license 4.0. http://creativecommons.org/licenses/by/4.0.)*

07/2009 [H1N1]). The trial involved 61 healthy volunteers aged 18–45 years, half of whom received the Nanopatch patch vaccinations in the volar forearm or upper arm, while 15 received intramuscular (IM) injections [68]. The researchers concluded that the Nanopatch is considered safe as an IM injection where the application site reactions were mild or moderate. Nanopatch vaccination also produced immune responses comparable to IM vaccination and was more well-accepted by immunization recipients [68].

Aside from Nanopatch, the MicronJet or the later version MicronJet600 devices developed by NanoPass Technologies Ltd. was another silicon microneedle device that has been through human clinical trials. The device can be attached to a standard luer-lock or luer-tip syringe. This allows drugs to be injected directly into the skin from any prefilled or disposable syringe [88]. The intradermal delivery device consists of three pyramidal-shaped hollow silicon microneedles of 600 μm in length. The manufacturing techniques of MicronJet have been previously explained in Section 3.1 [21]. So far, 10 clinical trials on humans utilizing MicronJet/MicronJet600 have been conducted (recently reviewed in [89]). These clinical trials evaluated the efficacy and

safety of the device in delivering intradermal drugs and vaccines. These include the delivery of 2% lidocaine hydrochloride saline (NCT05108714), adalimumab saline (NCT03607903), human C19A3 proinsulin peptide (NCT02837094), indocyanine green in 5% human albumin solution (NCT04393168), eight amino acids, three antioxidants acid, vitamin B6 and two minerals (NCT02497846), S-OIV H1N1 vaccine (NCT01049490), hepatitis B vaccine (NCT02621112), polio vaccine (NCT01813604 and NCT01686503), and trivalent 2010/2011 influenza vaccine (NCT01304563) [88,89]. In a randomized trial by Hung et al. [90] (NCT01304563), the safety and immunogenicity of the trivalent influenza vaccine between full-dose IM vaccination via BD Soluvia prefilled microinjection (Becton Dickinson and Company) and low-dose intradermal vaccination via MicronJet600 was compared in elderly and chronically ill adult volunteers. They found that the intradermal vaccination resulted in higher immunogenicity than the IM vaccination, possibly due to the shallow delivery of vaccines by MicronJet600 that reached immune cells in the skin [90].

5 Conclusion and future perspective

Silicon microneedles are a promising technology for drug delivery. They are minimally invasive and can be used to deliver drugs transdermally. This chapter briefly overviews the different types of silicon microneedles available, such as solid, hollow, coated, hydrogel-forming, and dissolving microneedles. In addition, we also delve into the manufacturing techniques used to create these needles, as well as the results of clinical trials conducted on animals and humans using silicon microneedles.

The field of silicon microneedles for drug delivery is an area of active research and development. These microneedles offer several advantages over traditional drug delivery methods, including improved patient compliance, reduced pain, and enhanced precision in drug administration. Here are some potential future perspectives for silicon microneedles in drug delivery based on current trends and possibilities [91].

1. Increased precision and control: With silicon microneedles, precise control over drug delivery is possible, enabling the administration of specific dosages at predetermined rates. With the advancement of technology, we can expect even greater precision, which is crucial for personalized medicine and the treatment of various medical conditions.

2. Expanded range of drugs: While initially, silicon microneedles were primarily used for delivering small molecules and vaccines, researchers are developing microneedles capable of delivering a broader range of drugs, including larger molecules such as proteins and peptides. This expansion opens up new possibilities for treating a wider array of medical conditions.
3. Integration with wearable devices: Integrating silicon microneedles with wearable devices provides real-time monitoring and control of drug delivery. This enhances the ability to adjust drug doses based on an individual's specific needs, improving treatment outcomes and minimizing side effects.
4. Biodegradable materials: With ongoing research, biodegradable silicon microneedles are under development. These needles are designed to dissolve or degrade after delivering the drug, eliminating the need for needle disposal and reducing environmental impact.
5. Smart microneedle systems: Future silicon microneedle systems may incorporate smart technologies, typically sensors and feedback mechanisms. These systems will monitor physiological parameters and adjust drug delivery in response to changes in the patient's condition, optimizing therapeutic outcomes.
6. Enhanced patient acceptance: Improvements in design and materials contribute to increased patient acceptance of silicon microneedles. Reduced pain, minimized tissue damage, and user-friendly designs make microneedle-based drug delivery more appealing to patients compared to traditional injection methods.
7. Regulatory approval and commercialization: As the technology matures and more clinical trials are conducted, regulatory approvals for silicon microneedle-based drug delivery systems will increase. This will lead to broader commercialization and widespread adoption in clinical settings.

In conclusion, silicon microneedles are a promising technology with a bright future in drug delivery. They offer advantages over traditional drug delivery methods and can be used to deliver a wide range of drugs transdermally. However, optimizing the fabrication process and enhancing the efficacy of drug delivery using microneedles requires more research.

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CHAPTER 6

Polymer-coated polymeric (PCP) microneedles for controlled regional drug delivery

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1 Introduction

Microneedles (MNs) are an array of micron-size needles with the length of the needles ranging from 100 to 1000 μm. MNs were first investigated for the delivery of therapeutic agents into and across the skin [1]. MNs create tiny pathways that allow drugs to penetrate across the epidermis and dermis. MNs have several exceptional benefits. Its benefits include rapid painless delivery of drugs and biologicals [2]. MNs are of different types, such as solid, hollow, dissolving, and coated. Recently, polymeric dissolving MNs gained a lot of visibility and interest because of their unique ability to hold more drugs than conventionally used metallic MNs [3]. Any damage caused to the skin barrier has been reported to recover between 2 and 40 h depending on the type of MN and region of application [4]. When the dissolving MNs penetrate the biological tissues, they become softer and disintegrate. Therefore, they are generally formulated with biocompatible polymers. The fact that there is no need for retraction of the needle array, no hassle of disposal, minimal risk of tissue sensitization, and better payload delivery renders it preferable over silicon and metal MNs [5].

However, dissolvable MNs are also associated with a few disadvantages too. The immediate release or dose dumping of the active pharmaceutical ingredient (API) could cause tissue irritation [6]. Another safety concern that must be investigated is the consequence of the deposition of polymers in tissues after MN application [7]. Most of the excipients have no role in the

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treatment. They are used to prepare a stable construct. The potential skin sensitization issues due to dumping of the API or polymer could potentially pose serious hurdles in the product regulatory approval process [8].

In the case of delivery of drugs into and across the skin, MN-mediated delivery is an approach to bypass the stratum corneum (SC) to enable the delivery of drugs into the viable tissue and epidermal-dermal junction. MNs could potentially treat tissue disorders, allowing for focused administration [9]. For example, MNs could be helpful for the effective treatment of acne, scars, and hyperpigmentation.

The drug would diffuse across the deeper layers along the concentration gradient and reach dermal circulation (paracellular diffusion) as well. The drug moiety could also undergo diffusion via tight junctions [10,11].

Likewise, the MN penetrates the upper mucosal layers and delivers the drug into the serosal/submucosal tissue layers. This might open up a new mode of treating mucosal disorders more effectively.

The rate of drug absorption from MNs into the blood can vary depending on the type of MNs used, the properties of the drug being delivered, and the characteristics of the target tissue [11]. The drug released into the tissue could get absorbed intracellularly and traverse along the intracellular pathway across the tissue. A third pathway involves lymphatic uptake, in which the drug is absorbed into the lymphatic system; primarily when the API is delivered in the form of liposomes, nano-, and microparticles [12,13]. The encapsulated drug formulations can improve the intracellular uptake of drugs and would be suitable for treating chronic skin disorders such as melanoma and other inflammatory conditions. The encapsulated dosage forms mediated delivery by MNs can mitigate the degradation of API in the target tissue [14].

As mentioned earlier, in the case of dissolvable MNs, the dumping of the API could lead to skin reactions in sensitive populations. Moreover, the delivery of polymers alongside the API could be unsafe. Several polymers used in the preparation of dissolvable MNs have been reported to cause local and systemic reactions. Polyvinylpyrrolidone (PVP) is one of the polymers used for the preparation of MNs to deliver drugs and protein therapeutics [15]. One of the studies reported that higher molecular weight PVPs administered via intravenous and intraperitoneal methods exhibited localization in the reticuloendothelial system (RES) [16]. Despite being a well-known food additive, pharmaceutical excipient, and cosmetic ingredient, PVP has drawbacks that prevent it from being used at specific concentrations. Certain allergic responses have been caused by PVP or its compounds

[17,18]. Crospovidone could potentially harm the pulmonary arteries [19]. Since it is chemically inert, enzymes cannot further degrade it in the tissues either [20]. It is likely that PVPs modulate the immune system and could sensitize people. Likewise, even a hyaluronic acid MN containing alendronate was reported to cause mild to moderate irritation due to hyaluronic acid in rats [21,22].

In general, the consequence of the deposition of the polymer in the tissues and potential reactions are not well investigated. Although polymers are used as a part of the topical formulations, generally their exposure is limited due to stratum corneum barrier.

2 Polymer-coated polymeric microneedles

As discussed earlier, dissolvable polymeric MNs are designed to disintegrate quickly in the tissue to deliver active ingredients. Therefore, there is no control over the rate of release of drugs. The drug release rate could be maintained if the dissolvable MNs are coated with a semipermeable polymer. The polymer-coated polymeric (PCP) MNs emerged as a potential approach to controlled release of API. Slower release may provide several advantages, including reducing drug-induced tissue irritation, improving local therapeutic activity, and maintaining therapeutic levels for a longer period [23]. In PCP MNs, the MNs are coated with a semipermeable polymeric layer that only permits the active ingredient to diffuse out of the MNs, preventing the polymer construct from dissipating in the tissue (Fig. 1) [24]. The PCP MNs are also used to extract the drugs and biomarkers from the skin tissue. The PCP MNs remain intact after being inserted into the tissue, allowing for the diffusion of drugs and analytes inside and outside the membrane [25].

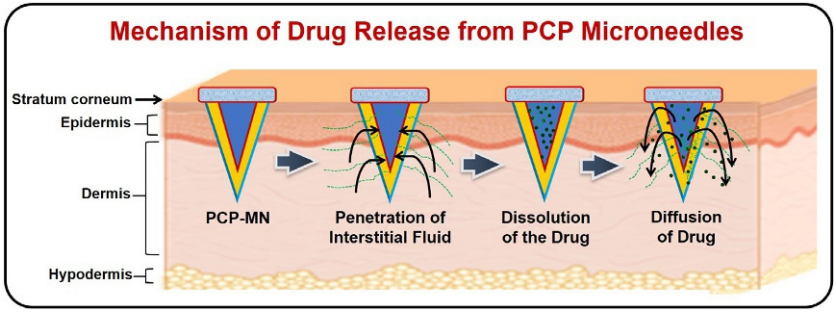

Fig. 1 Mechanism of drug release from PCP microneedles into the target site.

3 Preparation method of PCP microneedles

The polymeric MNs are made using a polydimethyl siloxane (PDMS) template. The polymer-coated MNs were prepared using the dip and spin technique. The core MN array was attached to the paddle shaft with the help of adhesive tape, dipped into the coating liquid, spun, and allowed to dry. Coated MNs were further investigated for coating intactness, microscopy, and release studies (Fig. 2).

4 Variables that could affect the coating uniformity and thickness

Viscosity: The viscosity of the coating polymer has a significant influence on the thickness of the coating and its mechanical properties. Higher viscosity affects the MN shape, and lower viscosity coating may spread very thin and will not be uniform on the MNs. Therefore, optimization of viscosity is a critical step in the coating process (Fig. 3).

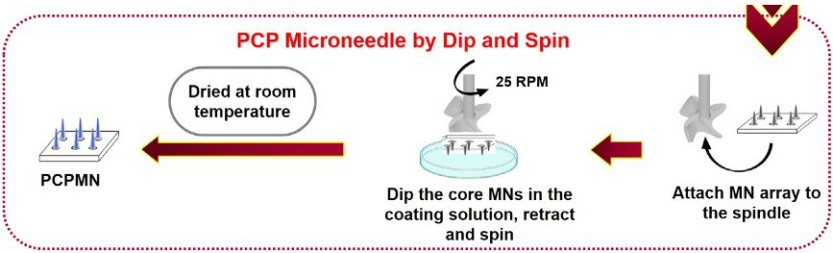

Fig. 2 The stages of preparation of PCP microneedles.

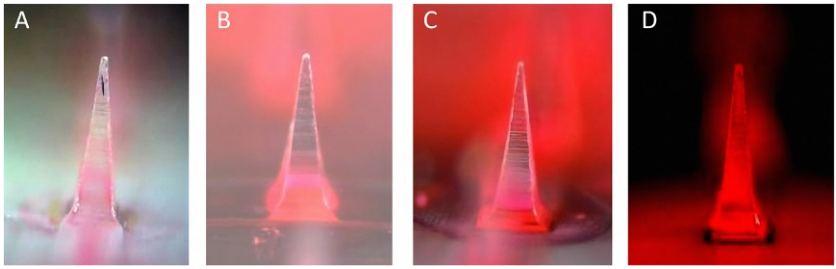

Fig. 3 Bright field microscopic images of PCP microneedles, coatings with different concentrations of polymer, (A) 1% w/v of EC, (B) 2% w/v EC, (C) 3% w/v EC, and (D) 4% w/v EC.

Solubility of the polymer: Ethyl cellulose and Eudragit are very good sources of water insoluble, sustained drug-release polymers.

Process variables: The coating's thickness and homogeneity may vary depending on the method of application. Various application methods include spraying, brushing, and roller coating, which could be explored. Each technique has a unique set of factors that might affect the outcome. In the case of the dip and spin technique, the variables that could influence the coating uniformity and thickness are rotation speed, duration of spinning, and drying rate. Optimal centrifugal force is necessary to achieve a uniform coating. Suboptimal spinning could lead to dripping of the formulation or tailing of the coating formulation to the tip. On the other hand, spinning at a higher rate than the optimal rate could lead to the escape of the coating solution from the surface due to high centrifugal force.

Operator skill and technique: The uniformity and thickness of the coating can be significantly influenced by the operator's expertise and application technique. Experience, training, and attention to detail are crucial to achieving consistent and homogeneous coatings.

5 Testing the integrity of the coating

Visual examination: Visual inspection could reveal any noticeable defects such as cracks, peeling off, or unevenness in the coating layer. However, more sophisticated methods are required to investigate the presence of pores and microstructural defects in the coat.

Mechanical strength test: Mechanical stress is applied to the coated MNs to investigate the effect of coating thickness on the mechanical properties such as break strength and penetrability into the tissue. One can use modified methods using texture analyzers and tensiometers for evaluating the MNs for mechanical properties [26].

Microscopic examination: The coated MNs can be examined for any defects on their surface and uniformity of coating, and morphometrics can be measured as well using optical microscopy or scanning electron microscopy (SEM).

Stability testing: Stability testing evaluates the coated MNs' stability and long-term performance. Over a predetermined period, the MNs are exposed to various environmental factors, including temperature, humidity, and light exposure. This testing assists in determining whether the coating maintains its consistency, and physical properties over time.

6 Microscopy

Characterization is crucial to the development of MNs of uniform morphometrics and mechanical properties and provides a qualitative and quantitative evaluation of MNs for a more appropriate structure [27]. MNs are mostly characterized by optical microscopy and SEM. Brightfield microscopy is an increasingly common technique for the optical microscopy of MNs. In this method, MNs are viewed using a microscope using visible light. This makes it possible to observe MNs' size, shape, and distribution of colored API (Fig. 4).

Scanning electron microscopy is commonly used to image MNs because of its high resolution and capacity to offer thorough surface information. The morphological characteristics of MNs, such as their height, tip, base diameters, spacing between them, base dimensions, and thickness of coating, are assessed using SEM. It also assesses surface characteristics like pores and roughness. The MN patch is mounted on the stage and coated with a gold solution using sputtering to improve visualization. The open-source ImageJ program can visualize images [27] (Fig. 5).

7 Coating intactness test using marker molecules

Drug release: The immediate release of the active components is one of the indications of a lack of coating integrity. One can use some macromolecules such as FITC albumin or FITC dextran, as well as marker molecules, to test the size-selective nature of the coating. The coating integrity test is conducted by adding FITC-albumin to the MNs. The IVRT study was carried

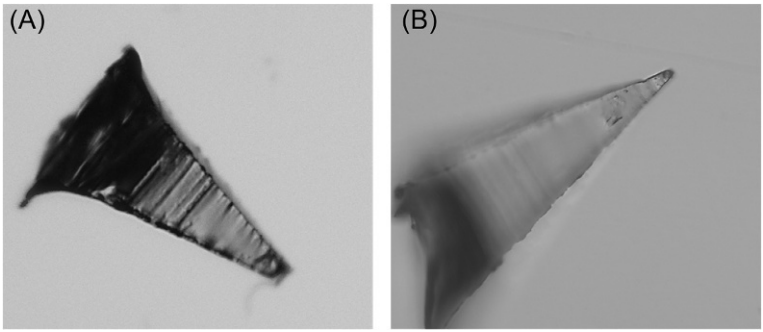

Fig. 4 Bright field microscopy of before (A) and after coating (B) (uniform surface) of polymeric microneedles, intentionally detached from the array to capture the morphology of microneedles.

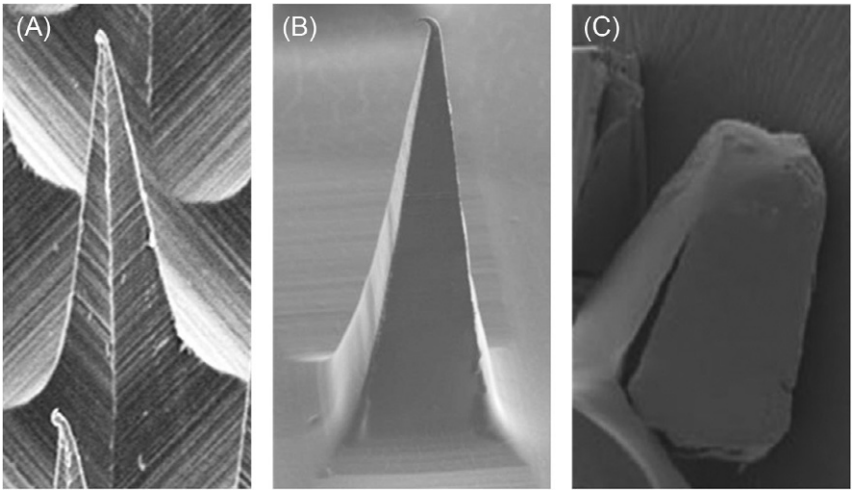

Fig. 5 (A) Scanning electron microscopic (SEM) image of the uncoated microneedle, (B) the coated microneedle with a uniform coating layer, and (C) outer polymeric coating is captured. (The coating was intentionally separated from the microneedle.)

out to evaluate the coating integrity of PCP MNs. For 6 h, the coated MNs are submerged in the receiver medium, pH 7.4 phosphate buffer saline (PBS), in such a way that only the needles are in contact with the buffer, not the backing membrane. The buffer was taken out at regular intervals, and the same amount of buffer was replaced. The samples are read by using UV fluorospectroscopy, and their fluorescence content is examined. The PCP MNs that caused visible fluorescence in the buffer are regarded as faulty. Aiming for zero fluorescence in the buffer media is essential to maintain coating integrity. To achieve an intact coating, the process factors and coating formulation variables were adjusted [24,28–30].

8 In vitro release testing (IVRT)

After the PCP MNs pass the integrity test, the MNs are subjected to a test for dye release testing. A custom-made setup in vitro release investigation of the PCP MNs was carried out. The PCP MN array (single array) was mounted on a movable string, so that only the needles were dipped into the buffer (PBS 7.4) and not the backing platform. The receiver media in the beaker was 5 mL of the buffer. The temperature was kept at 32°C while the buffer was agitated at 100 rpm. 200 μL of samples was taken at various time intervals. At each sampling, an equal quantity of buffer was replenished (Fig. 6).

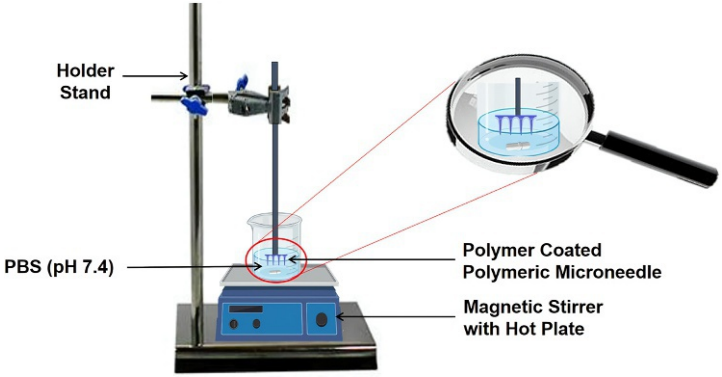

Fig. 6 Custom-made apparatus setup to conduct the IVRT studies of PCP microneedles. The enlarged image shows only the needles in contact with the buffer medium.

9 Applications of PCP MNs

(a) **Intravitreal space:** Polymer-coated MNs prove to be an excellent choice for controlled delivery of drugs using MNs. Intravitreal injections are known to cause severe side effects and a surge in the intraocular pressure, leading to extreme discomfort. Moreover, as discussed earlier, delivery of excipients that do not have any therapeutic value into sensitive tissues like the eye could be a potential concern. Therefore, there is a need for a minimally invasive drug delivery system that results in a slow infusion of only the drug (without dumping solvents and excipients), making it a safer and relatively more patient-compliant option when compared to conventional intravitreal injection. PCP MNs can be a valuable alternative to intravitreal injections from a safety, efficacy, and patient compliance standpoint (Figs. 7 and 8).

(b) **Skin (Melanoma):** Constant long-term exposure to chemotherapeutic and immune-modulating drugs can be relatively more efficacious therapeutically than bulk administration to the affected site. The use of biodegradable polymers to achieve controlled delivery in indications would not be feasible due to the very slow degradation kinetics of the polymer (days to weeks). PCP MNs is a promising approach of delivering drugs into the dermal tissue over a few hours. The micropores recover rapidly, and the skin will be ready for subsequent administrations (Figs. 9 and 10).

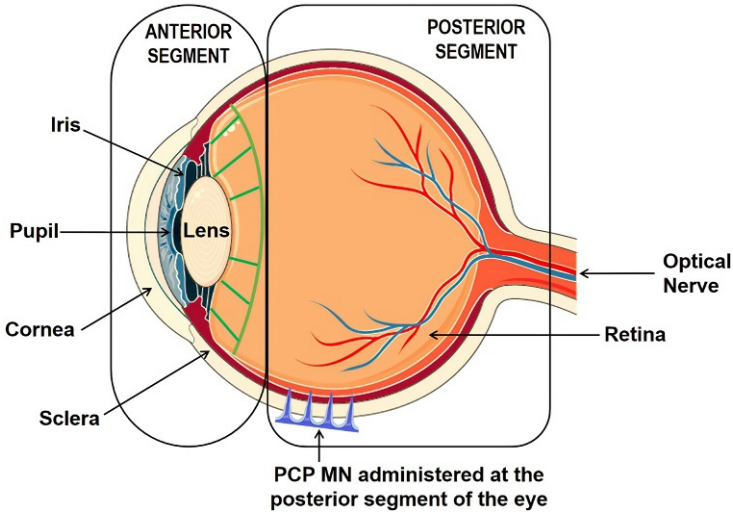

Fig. 7 PCP MN application to the posterior segment of the porcine eye to evaluate the intravitreal delivery of drugs.

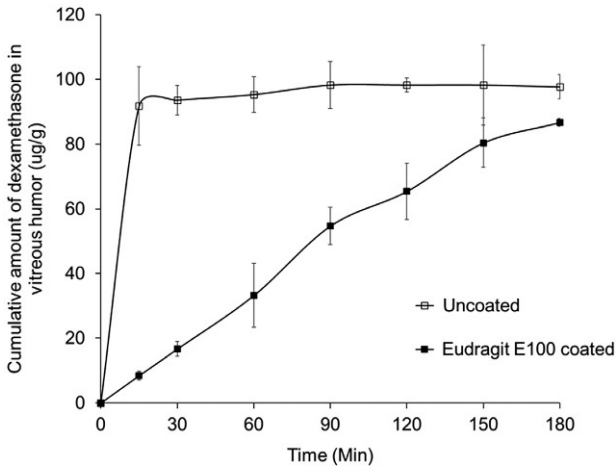

Fig. 8 Intravitreal delivery of dexamethasone release data profile compared between uncoated vs. Eudragit coated MNs.

The research on PCP MNs is continued to discover their application in different indications. A few are the following:

1. Sampling the extracellular fluid's components from the tissues (Fig. 11).

   The absorbent entrapped in PCP MNs was captured under bright field microscopy. The left-side picture is the polymer MN, and the right-side picture is a single PCP MN with adsorbent entrapped [24].

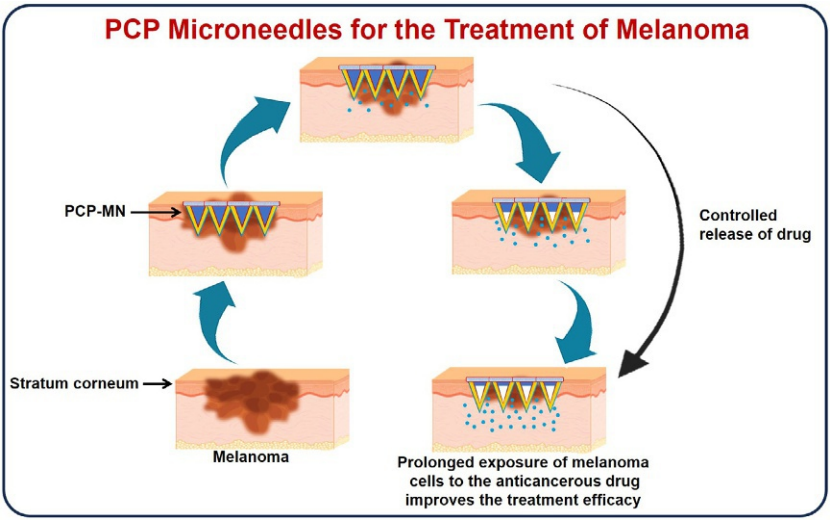

Fig. 9 Figure showing the release of drug 5 FU in the skin tissue.

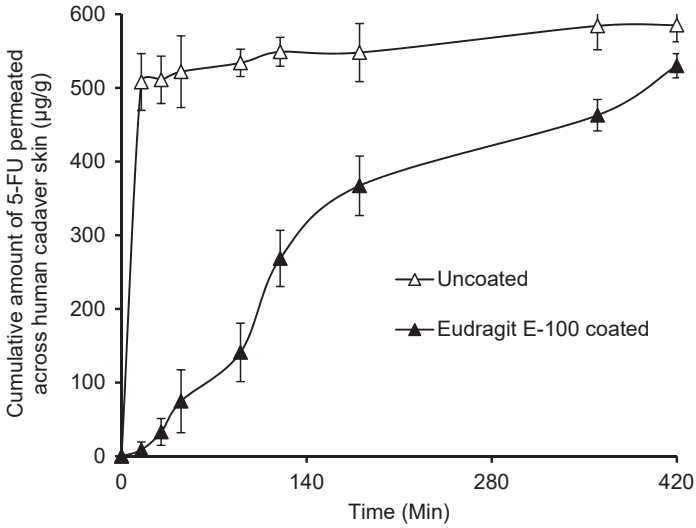

Fig. 10 Cumulative amount of drug permeated across human cadaver skin.

2. Therapeutic drug monitoring and determining the locoregional kinetics of drugs after topical or systemic administration.
3. An approach to achieve a controlled drug release at the site of application.

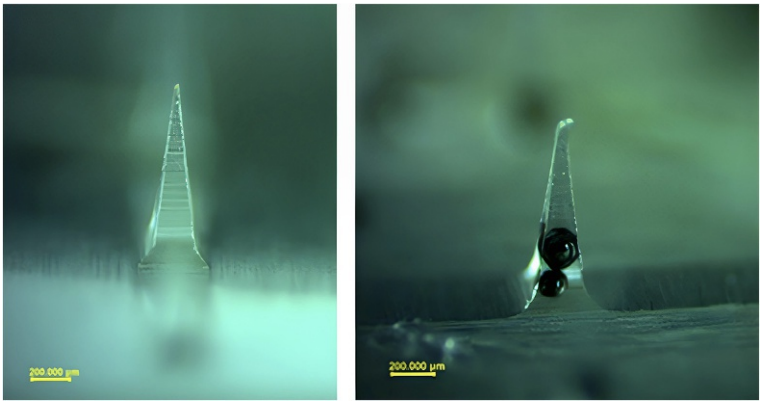

Fig. 11 Adsorbent entrapped in a PCP microneedle for sampling of contents from the extracellular fluids from tissues.

4. An approach for the delivery of drugs into mucousal tissue, and sensitive organs like back of the eye.

10 Conclusions

PCP MNs is a novel approach in which soluble MNs can be rendered safer by allowing only the drug to diffuse from the device. This would mitigate any untoward consequences owing to potential accumulation of the polymer in the tissue. The approach would be useful for the slow release of drugs into the tissue for the treatment of local or systemic disorders. The release rate could be modulated by varying the coat thickness and polymer grade used to make the core MNs.

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CHAPTER 7

Microneedles for local and transdermal drug delivery

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1 Introduction

The transdermal route of delivery is an attractive, noninvasive approach for drugs that are unsuitable for administration via the oral or enteric routes [1]. The transdermal pathway offers a painless approach to deliver drugs into the blood vessels located in the dermis, while bypassing the hepatic first-pass effect [2]. The presence of the surrounding subcutaneous fat tissue can also serve as a reservoir to sustain drug release. Transdermal drug delivery systems have therefore gained wide acceptance for use worldwide. However, transdermal drug permeation is limited by low diffusional flux through the highly hydrophobic stratum corneum. Transdermal drug absorption is therefore limited to highly potent, small molecular weight drugs with high log *P* values.

Microneedles are transdermal drug delivery devices that have emerged as a promising technology during the last two decades. Microneedle patches (MNPs) possess the advantages of conventional transdermal drug delivery systems, while overcoming the restrictions posed by dosing levels, molecular size, and diffusional flux. A microneedle device consists of a patch of miniature needles with lengths that are sufficient to penetrate the stratum corneum; these are typically in the range of 250–300 μm [3]. The microchannels created in the stratum corneum are reversible [4], predominantly due to the fluidity of the lipoprotein matrix constituting the stratum corneum. They may be used for a wide range of clinical applications such as psoriasis [5–13], opioid overdose [14,15], analgesia [16,17], diabetes management [18–22], wound healing [23–27], vaccine administration [28–36], and diagnostic monitoring [37–42]. MNPs may contain arrays of solid [43–46], coated [47–49], hollow [50–56], degradable [57–62], or dissolvable [63–68] microneedles.

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2 Local drug delivery

2.1 Psoriasis

Psoriasis is a chronic skin condition resulting from the hyperproliferation of keratinocytes and frequently manifests as thickened, inflamed skin with scaly patches [6]. While oral or parenteral administration of methotrexate can significantly relieve symptoms, the high doses associated with systemic therapy have necessitated researchers to explore other drug delivery approaches to maximize local target concentrations. Numerous groups have recently demonstrated the feasibility of methotrexate administration via microneedles to relieve psoriatic symptoms in mouse models [6,11–13]. Wang et al. used supramolecular dissolving MNPs composed of hydroxypropyl beta-cyclodextrin to load and efficiently deliver triamcinolone acetonide into the ear lesions of imiquimod-induced psoriasis-like mice [5]. Hyaluronic acid-based dissolving microneedles have also been explored for the delivery of daphnetin [9] and methotrexate [13] to treat psoriasis.

2.2 Wound healing

The treatment of chronic wounds with systemic long-term antibiotics risks antimicrobial resistance, high side-effect profile, and poor efficacy. Long-term wound treatment may result from instances of trauma and burns, while diabetic wounds represent a more chronic condition that can escalate to the level of amputation, if not addressed appropriately. Yang et al. have recently demonstrated that multicomponent hyaluronic acid microneedles embedded in a cerium/zinc-based nanomaterial can accelerate wound healing in diabetes wounds by destroying the oxidation balance of the bacterial pathogens present in the wound [69]. Other drugs delivered through a microneedle approach for wound healing include moxifloxacin [70], tetracycline hydrochloride [27,71], polymixin [72], deferoxamine [71], magnesium hydride [73], recombinant human epidermal growth factor (rh-EGF) [27], vascular endothelial growth factor (VEGF) [72], trichostatin A (TSA) [74], and even insulin [75].

2.3 Local analgesia

Microneedles have the advantage of delivering higher dosages of anesthetics compared to topical creams or lotions. However, the minor epidermal injury created due to microneedle penetration has to be considered as an important factor for local analgesia treatment. Zhao et al. used gelatin-methacryloyl microneedles to sustain the delivery of lidocaine hydrochloride and test for

pain sensitivity in spared-nerve injury (SNI) rats [17]. They report that these microneedles are safe and effective to provide longer lasting local analgesia.

3 Systemic drug delivery

3.1 Insulin delivery in diabetes

Diabetes is a major metabolic disorder in the 21st century, which is impacting over 280 million people across the globe [76]. The standard treatment practice for type 1 diabetes includes frequent monitoring of blood glucose and subcutaneous insulin injections [77], which are painful. Improper insulin dose titration can cause hypoglycemia, and consequences can be lethal. Therefore, a smart insulin drug delivery device that can autotitrate insulin administration based on physiological glucose levels would be ideal to address this unmet need. Researchers started evaluating microneedle-based insulin patches with the feedback loop for blood glucose monitoring. Insulin release can be controlled with glucose binding protein [78] (Con A) glucose oxidase,[79] and phenylboronic acid-based glucose monitoring systems. Translatability for the Con A-based insulin delivery system is limited owing to its instability and toxicity in vivo. Glucose oxidase-based insulin patch has glucose-responsive vehicles (GRVs) encapsulated in hypoxia-sensitive hyaluronic acid complex (HS-HA) needle material [79]. GRVs are self-assemblies and contain Gox and insulin. Under hyperglycemic conditions, glucose undergoes enzymatic oxidation, resulting in local hypoxia, which reduces the HS-HA complex to release GRVs and insulin from the needle. When glucose levels are normal, the feedback loop for HS-HA complex reduction is stopped, so insulin will no longer be released from the microneedle system.

3.2 Opioid overdose

Naloxone is an opioid inhibitor and has found wide applicability in reversing overdose complications. Based on the condition of the patient requiring treatment, nonoral routes providing rapid action, such as intramuscular or intranasal administration, are preferred [14,15]. Puri et al. have performed modeling simulations that suggest that the pharmacokinetic profile of naloxone delivered through the microneedle approach may be comparable to those obtained with FDA-approved intramuscular and intranasal devices [14].

3.3 Systemic analgesia

Song et al. have used a microneedle approach for the systemic delivery of ziconotide-loaded liposomes for chronic pain treatment. Xie et al. have demonstrated a safe and effective approach to treat localized neuropathic pain to transdermally deliver calcitonin gene-related peptide (CGRP) via dissolvable microneedles [80].

4 Vaccine administration

4.1 Types of vaccines

Vaccines can be broadly divided into preventive or therapeutic categories based on their application. A therapeutic vaccine is given to sick people to help their immune systems fight illness, and a prophylactic vaccine is used as a form of preventive medicine to prevent infection. Historically approved vaccines are either whole pathogens (inactivated and live attenuated), subunit vaccines, or nucleic acid vaccines [81]. The use of vaccination as a preventative measure against infectious diseases and as a medical tool to control pandemics around the world has proven to be successful in the past (Fig. 1).

The most recent COVID-19 pandemic is a classic example of how a pandemic can be managed via vaccination, thanks to international health agencies for emergency approval of COVID vaccines to reestablish public health normalcy. From a drug delivery perspective, lipid nanoparticles (LNPs) have emerged as a major nonviral vector platform during the pandemic and a hypodermic needle was used to inject vaccines under the skin. A licensed healthcare professional and a clinical setting are required for vaccination. The pandemic exposed weaknesses in the global healthcare system, which consists of the pharmaceutical manufacturing infrastructure and supply chain, immunization, and urgent care center capacity. Although lipid nanoparticles were previously commercialized, vaccine manufacturing for humanity across the globe resulted in a very high demand for lipids worldwide, and a shortage of providers created a bottleneck in the market. Further, frozen DP storage requirements were a barrier to vaccine distribution, delaying vaccination in developing nations. To address the above-stated concerns, microneedles are actively evaluated for vaccination, which is in the early stages of development.

A microneedle array of coronavirus-subunit S1 by Kim et al. demonstrated good immunogenicity in mice. Other infectious disease indications

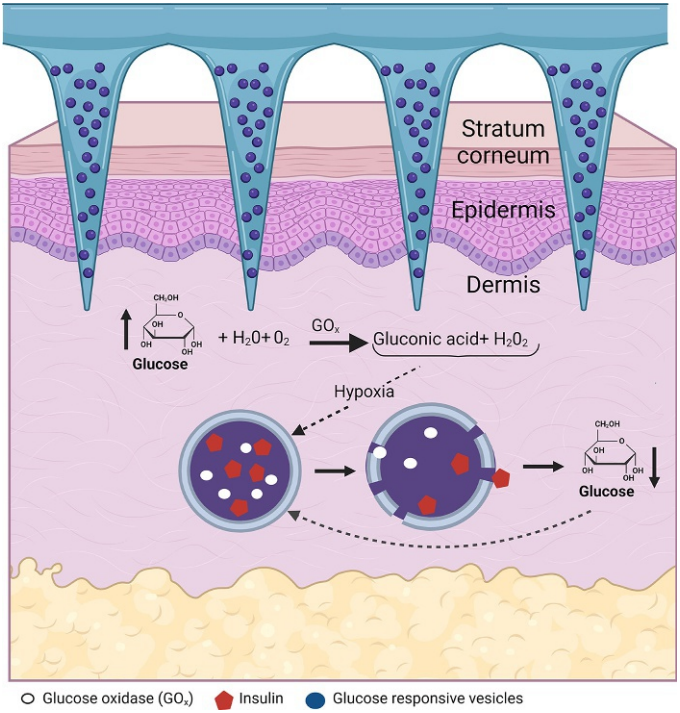

Fig. 1 Microneedle-based insulin delivery.

such as hepatitis B and influenza were evaluated for dermal MNP delivery [82]. Choi et al. developed a dissolving microneedle patch for hepatitis B, which elicited an immune response in mice and rhesus monkeys [29]. Joyce et al. developed a measles and rubella microneedle patch, which was immunogenic in infant rhesus macaques [83]. Trivalent influenza TIV-MNP 2015 was tested in a phase I clinical trial where a control group received intramuscular administration and the therapeutic efficacy of microneedles was assessed [84]. This study included self-administration of the MNP by subjects in addition to healthcare professional administration. Overall, the influenza MNP was well tolerated and immunogenic, and it validated the MNPs' capacity for self-administration. Ellison et al. developed a VaxiPatch to deliver rHA of influenza virus B/Colorado/06/2017 [85]. The future holds promise based on the preclinical performance of microneedles in animal models. The mechanism of action for microneedle immunization is described in the following section.

4.2 Mechanism of immunization

Skin is the largest organ in the body and is immunocompetent, which makes it an active target for vaccination [86]; however, stratum corneum acts as a barrier for accessibility to the immune system. Invasive techniques such as SC and IM injections are successful in delivering therapeutic agents in the deeper layers of the skin or muscle but are painful to the patient. Needle-phobia is a reason for poor patient compliance [87]. Microneedles on the other hand can be used to administer vaccines and have a great potential to evoke immune response and are minimally invasive [88].

Microneedles can deliver vaccines to dermis region, which is rich in antigen-presenting cells (APCs) such as dendritic cells, macrophages, and T cells [89]. Upon administration, pathogenic recognition receptors (PRRs) detect antigens, and naïve dendritic cells (DCs), a form of antigen-presenting cells (APCs), are activated and drain to the lymph node. Further immune cascading reactions result in the release of signal transducers such as adaptor proteins, protein kinases, transcription factors, inflammatory cytokines and chemokines. Through integrin expression, inflammatory mediators control leukocyte recruitment. When a pathogenic infection occurs, the interaction of MHC-II with CD4 + T cells causes T cells to differentiate into T-helper (Th) cells. B-cell maturation into plasma cells, which release antigen-specific antibodies, is induced by Th cells. In order to respond to upcoming infections, a subset of matured B cells develops into memory B cells (Fig. 2; Table 1).

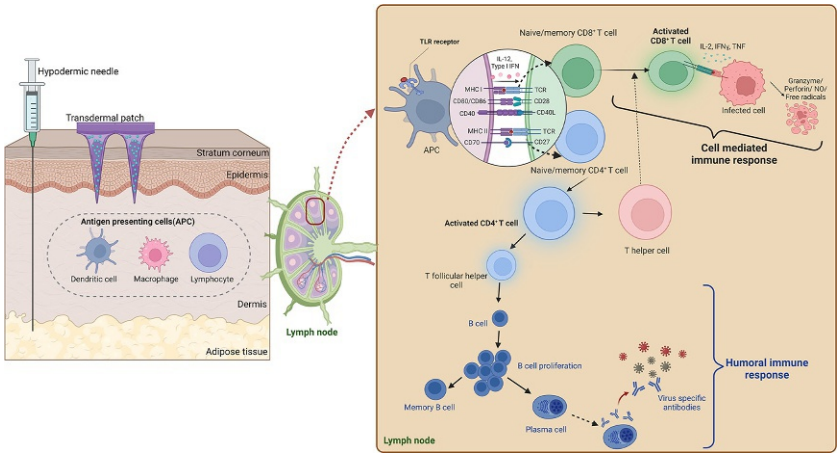

Fig. 2 Immunization mechanism for microneedles.

Table 1 Microneedle-based drug development.

Company	Application	Indication	Development status	References
CosMed transdermal drug delivery	Cosmetic	Wrinkle treatment	FDA approved	[90]
Sanofi Pasteur	Prophylactic	Influenza and Hepatitis B	FDA and CE approved	[91]
Quad medicine		4-in-1 influenza, 5-in-1 (DTP-HepB-Hib), osteoporosis, and alopecia	Preclinical	[92]
Vaxess		Influenza, COVID-19, and skin cancer	Booster Ph1	[93]
Mimix		RSV	Phase I	[94–96]
		Dengue	Phase II	
		Enterovirus EV71, MVC COVID-19, and influenza quadrivalent vaccine	Phase III	
Nanovax		Flu vaccine	Clinical studies completed	[97]
Skinject	Cancer (Doxorubicin)	Anticancer	Phase II	[98]

5 Advantages of microneedles

The passage of drugs through the transdermal route provides direct access to the blood circulation. Microneedles allow drugs to directly enter the systemic circulation, which helps for better bioavailability, rapid absorption, and efficacy. However, this passage of drugs through the transdermal route is restricted due to the presence of stratum corneum. Molecules over 500 Da with high lipophilicity cannot cross the stratum corneum. This limitation of traditional transdermal drug delivery is addressed by microneedles. Microneedles provide numerous advantages over conventional transdermal drug delivery systems. The unique geometry of microneedles allows direct access to the dermis bypassing the stratum corneum barrier. They provide a passage for the drugs through the stratum corneum or create microscopic pores into the skin from which large molecules can be absorbed [99]. For instance, insulin could be delivered intradermally using a hollow microneedle as shown by Davis et al. Hormones that cannot be delivered using traditional transdermal systems were shown to be effectively delivered through microneedles [100]. Not only a wide range of molecules are delivered through microneedles, but these systems also offer painless drug delivery [101].

The micron size of the needles allows minimum to no pain during the delivery of drug. Generally, microneedles are fabricated to a desired needle height of 150–1500 μm [102]. This specific microneedle length allows the penetration precisely into the dermis and at the same time ensures that no nerve endings in the dermis are stimulated. The increase in the length of the microneedle has a direct impact on pain. Gill et al. [103] showed a 150-μm long microneedle was deemed painless when inserted in human volunteers, against a 26-gauge hypodermic needle [103]. A microneedle with a 0–200 μm length when inserted into the skin was found to be painless. The painless nature of microneedles is an important consideration, especially when it comes to patient compliance.

The additional advantage of its micron size is faster skin healing at the site of insertion compared to a hypodermic needle [104]. Studies have shown that microneedle-treated skin recovered its barrier properties from 2 to 40 h after application, depending on the length of the microneedle used [105]. This quick recovery process of the skin is beneficial to ensure minimum microbial penetration [106]. Additionally, as a microneedle punctures the epidermis, there is no direct access of the needle or external environment to the blood circulation. Microneedling can also reduce tissue trauma and cutaneous complications that can be caused by a hypodermic needle. Use of microneedles can also help against the fear of needles in patients [107].

Microneedles are micron-sized needle devices that are designed in multiple ways, making them easy to use. For instance, solid microneedles are available on rollers (Dermarollers) to be easily rolled onto the skin to make pores [108]. Dissolving microneedles are available as a patch, which when simply applied on the skin will insert microneedles, while hollow microneedles can be attached to a typical syringe, making it easy to inject drug solution through its bore [109]. These applications are easy and do not need special training or expertise when compared to an intradermal injection.

Microneedles can enhance drug efficacy as they directly deliver drug into the dermis. Drugs don't have to diffuse through a rigorous path as in the case of intramuscular or subcutaneous injections. The enhanced drug efficacy also helps in dose reduction. For instance, influenza vaccine administered to healthy volunteers through ID injection showed the same efficacy at half the dose when compared to IM injection, while microneedle-mediated hypoglycemic effect in rats was similar when compared to subcutaneous injection [110,111].

The drug delivery rate can be controlled effectively with microneedles when compared against traditional transdermal systems. In conventional transdermal systems, a drug has to diffuse through multiple layers of bases from diffusion through its own matrix, diffusion through the stratum corneum, through the layers of the skin, and into the circulation [112]. In most cases, the diffusion for these systems is membrane controlled where the stratum corneum is the rate-limiting step. In the case of microneedle drug delivery, a better control of drug delivery is possible as the rate-limiting step of the matrix, which can be controlled by changing formulation [113].

Microneedles with a modified surface like pocketed and grooved microneedles can be used for the targeting of drugs to a specific depth in the skin, which can load a greater amount of drug onto the microneedles [99]. Gill and Prausnitz made pocketed microneedles and controlled filling capacity, coating, and targeted drugs to specific lengths into the skin [114], while Han et al. showed that microneedles with grooves filled with drugs improved the delivery of antigens. A higher antibody response was observed as more antigen could be delivered in comparison with smooth microneedles [115].

Microneedles can also be used with other transdermal techniques to increase their efficiency. For instance, applications of methods such as iontophoresis [116], sonophoresis [117], and electroporation [118] have been explored. Chen et al. used iontophoresis along with microneedles to study the delivery of insulin unilamellar nanovesicles [119]. In a separate study, they also evaluated the use of microneedles with sonophoresis where the rate

and extent of delivery of calcein (623 Da) and bovine serum albumin (66.430 kDa) could be increased [120]. Electroporation has shown the most promising results when it comes to combination therapy. Each microneedle behaves as a microelectrode for electroporation because of which the need for electrodes is ruled out [121]. Micropumps provide precise delivery of drugs. Pumps control flow rate and pressure for the delivery of concentrated drug solutions especially in the case of hollow microneedles. A more accurate and precise delivery and fluid extraction was shown by Zahn et al. with microvalves and micropumps. Drugs were shown to be delivered precisely in response to metabolite levels [122].

6 Challenges

Although microneedle drug delivery is a rapidly growing technique, it has its own challenges when it comes to development, delivery, and commercial aspect. Each microneedle type presents its own challenges as summarized in Table 2.

The geometry of a microneedle plays a very important role in its delivery and also presents the highest number of challenges while fabrication. The insertion and penetration of microneedles are strongly dependent on several parameters, such as aspect ratio (height to base), length, tip, and distance between microneedles [131,132]. For instance, blunt-tip microneedles require a relatively high insertion force [133]. To achieve a desired depth, microneedles with sharp tips are recommended. However, an increased tip sharpness reduces the structural strength of the microneedles, rendering them more fragile. Fragile tips can pose a risk of the tip breaking off and remaining within the skin upon removal of the patch. In the case of microneedles made of metals, traces of metal might be retained beneath the skin, which may lead to irritation, erythema, swelling, discoloration, or other side effects [134].

Table 2 Microneedle types and their challenges.

Microneedle type	Challenge	References
Solid microneedles	Cracking and breaking	[123,124]
Dissolving microneedles	Drug loading and fabrication of selected molecules	[125,126]
Hollow microneedle	Back pressure from dermis and clogging of bore during delivery	[127,128]
Coated	Low dose due to limited surface area	[129,130]

Microneedle base to height ratio also impacts the delivery. For instance, longer microneedles have a better potential for skin penetration and drug delivery precisely into the dermis of the skin. However, the longer the microneedles, the more fragile the structure. Longer microneedles have shown fracture during not only drug delivery but also during fabrication [103]. To ensure structural strength, microneedles with a broader base can help increase the height of microneedles. However, this may create a microneedle array in which the interdistance between each microneedle is significant. This can not only impact skin penetration but also affect the delivery of drugs under the skin [135]. More tightly packed needles by reducing the aspect ratio can avoid skin penetration at all costs by causing bed-of-nails effect.

The highly immunogenic nature of the skin is very favorable for microneedles. However, it also may develop mild and temporary erythema depending on the size, substance, and type of the microneedle. A special attention toward the material of construction is also needed. Using the current fabrication techniques, there are a lot of polymeric options to select from. However, each material before use has to be evaluated for cytotoxicity, sensitization, irritation, and intracutaneous reactivity tests. Genotoxicity and subacute/subchronic systematic toxicity tests are additionally recommended for the latter period of use. The use of biodegradable microneedles is beneficial as they degrade and are removed from the skin. Most of the biodegradable polymer microneedle materials use either solvent or heat during the fabrication process. Both procedures are limited in the capability of working with large molecules. As microneedles have the most use in large molecules, the fabrication process that ensures the stability of large molecules like proteins should also be considered [136].

To ensure the stability of a large molecule, microneedles are first fabricated followed by coating a solution of protein [137]. A coated microneedle device can only deliver a very limited amount of drug. Another approach used to deliver large molecules is the use of hollow microneedles. Although hollow microneedles allow for high doses, continuous injection into the dermis bore may be obstructed by compressed skin tissue after microneedle insertion [106]. Microneedles can be only used for drugs that have low doses. The dosing capability of microneedles is limited due to its micron size. Even though hollow microneedles can deliver a higher amount of drug payload compared to other microneedle types, the dose is limited due to the thickness of the dermis.

Economic evaluations of the microneedle technology should also be considered. It can be predicted based on the complex fabrication, storage,

delicate nature, and long approval process, as with every new technology, the clinical use of microneedles can be comparatively expensive. Even though they show promise in research, their economic implications have yet to be calculated carefully [138]. To add to the cost, one must consider regulatory aspects as well. For instance, microneedle patch sterilization is another challenge that should be considered for commercial applications. If sterilization is deemed necessary, then the method of choice will be critical based on the drug used [139,140].

US FDA has always been concerned when it comes to combination products, particularly in stability testing, content uniformity, risk analysis, sterility validation, and manufacturing. In order to get an FDA approval, microneedle technology has to go through clinical application scenarios, which should be thoroughly proved using animal testing and clinical trials. To add to the cost and complexity, the submission process to the FDA for approval is not straightforward because submissions should be in the form of combination products that use microneedles. As per the FDA, combination products must consider the safety and effectiveness associated with the product as a whole [141]. FDA has adopted product-specific approval rather than microneedle design-specific approval. This process causes delays in approval, thereby restricting the commercialization of microneedles. Due to the possibility of high development cost and prolonged FDA review with slight chances of approval, only big pharmaceutical companies can invest in such a venture.

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CHAPTER 8

Silicon microneedles for transdermal drug delivery

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1 Overview

Over the last two decades, there has been considerable effort directed toward the development of structurally robust, low-cost, and biocompatible microneedles for dermal as well as transdermal drug delivery [1]. It is envisioned that these microneedles will be incorporated into a "lab-on-chip" (LOC) system designed for fluid testing, after which the results will be automatically communicated to medical professionals [2]. For drug delivery, different types of microneedles—either hollow or solid—can be employed, depending on the nature of the fluid transfer process. For solid microneedles, which are significantly simpler and cheaper to produce and which are the major focus of this chapter, there are several approaches: Firstly, solid microneedle arrays can be used to generate micropores on the skin and then a patch containing the drug is applied on the area so that the drug within the patch is diffused through the skin [3]. In an alternate approach, the drug can be contained within the microneedles, so that after the puncture the drug can be released. For this purpose, dissolvable or porous microneedles are necessary [4]. For solid microneedles without any porosity, the drug can be coated on the tip and delivered through the skin after piercing. After that, the chemicals are released through hydration of the coating. Finally for hollow microneedles, the drug is used in solution form, and through the bore of the microneedles, the drug is delivered actively or passively within the human body [5].

A schematic showing different types of microneedles described previously as well as the various layers of the skin is shown in Fig. 1. The three layers of the skin are stratum corneum, nonviable, and viable epidermis, and the total thickness of these layers may be up to 1500 μm. Thus, if drug delivery is to be carried out into the epidermis, the microneedle size should be approximately 1500 μm, sufficient to deliver the different types of the drug

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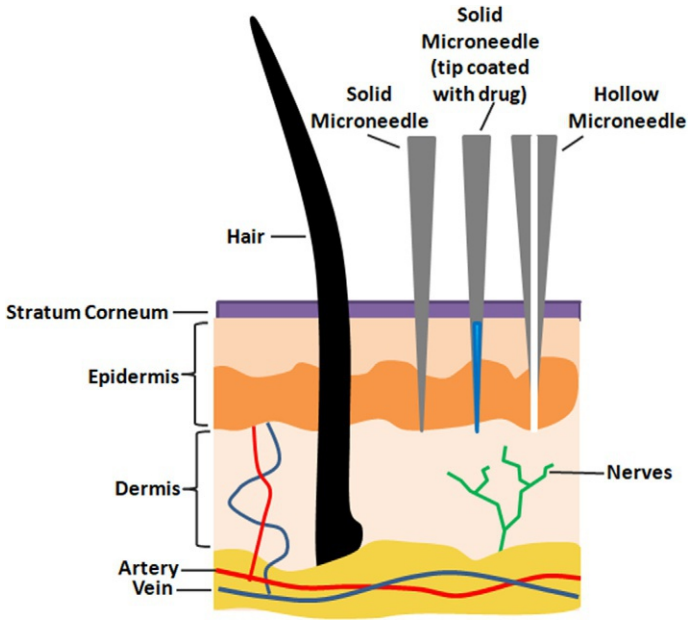

Fig. 1 Schematic of the various skin layers of the human body and different types of microneedles.

in the human body through the human skin. For transdermal delivery, ideally the microneedles should be 150–1500 μm in length and 50–200 μm in width, with a tip diameter of 1–25 μm. Metals can be used for microneedles due to their biocompatibility, but such needles cannot be degenerated in a biological environment. Semiconductors are used to fabricate solid, hollow, and coated microneedles. The disadvantage of these materials is that their residues within the skin may create side effects. Polymers that may be biocompatible as well as biodegradable can be used as a dissolvable-type material for the fabrication of microneedles, thereby reducing residues and related reactions.

2 Review of microneedles and fabrication techniques employed

Fabrication techniques developed by the microelectronics industry have found interesting applications in the development of microneedles. The standard microneedles are structures with diameters 2–25 μm and lengths of 300 μm–1 mm [6,7]. For withdrawing body fluids, the upper layer of the skin is pierced by these structures. Thus the drugs are also injected

painlessly using such structures. Furthermore, chip-level integration can be done within LOC systems. In order to carry out the processing of the fluids, microchannels are necessary to transport them to various components of the LOC system.

There are different approaches of drug delivery by hollow and solid microneedles. The solid microneedles can be used to deliver drugs into the skin; the three main mechanisms for drug delivery are the "poke and patch" approach, the "poke and release" approach, and the "coat and poke" approach. In the "poke and patch" approach, microneedles are used to generate micropores in the skin; after that on top of these micropores, a patch is applied to allow the drug in the patch to diffuse through the skin. The dissolving microneedles or porous microneedles are employed in the "poke and release" approach. Here the drug is released by dissolution of those microneedles within the skin. The final approach is the "coat and poke" method where the tip of the microneedles is coated with the drug. After poking the needles on the skin, the drugs are released automatically from the tips and diffused through the skin. The "poke and flow" approach is applicable to hollow microneedles. In this approach, a drug is delivered in solution through the bore of the microneedles actively or passively.

A combination of microneedling and microdermabrasion, along with light emitting diode (LED) therapy and simultaneous deep tissue serum infusion, is an important part of the latest and modified dermatological treatment known as DermaFrac treatment. Different dermatological conditions like aging and sun damage of the skin, uneven skin tone, fine lines, wrinkles, acne, enlarged pores, hyperpigmentation, and superficial scars are treated using this method. However, the microneedle parameters are problem specific; for example, a needle length of 1.5–2 mm is used for treating acne, whereas a 0.5-mm or 1.0-mm-long microneedle is preferred to treat aging skin and wrinkles.

For the fabrication of solid and hollow microneedles, different types of materials are used like polymers [8–10], metals [11], and semiconductors [12]. An important tool for the fabrication of such structures is silicon micromachining. For the silicon micromachining, reactive ion etching [13] or the wet chemical etching process is employed. The solid microneedles with a porous tip [14] were fabricated by several groups. In these microneedles, the drugs can be stored in the tip before delivery. Due to the nontoxicity and biocompatibility, porous silicon is used in microneedle fabrication [15] and the pore sizes can be varied in a wide manner [16–18]. But porous silicon microneedles are easy to break and they may stay within the skin after

piercing. The porous tip or the tip with microcavity is very useful for transdermal drug delivery. Such microneedles should also have a sharp tip so that they can pierce the skin. In this chapter, the low-cost process for the development of silicon microneedles with microcavity on the tip has been investigated. Here microcavities are generated spontaneously on the tip. A combination of isotropic [19] and anisotropic wet chemical etch processes are employed to fabricate such structures.

3 Silicon micromachining

Silicon bulk micromachining techniques have been used for more than four decades and are the most popular fabrication technology [20] for sensors such as pressure sensors and accelerometers. Silicon micromachining technology is important in the fabrication of microfluidic and nanofluidic chips, MEMS/NEMS, and other miniaturized devices [21].

Wet silicon micromachining (etching) is a material removal process that uses liquid chemicals or etchants to remove materials from a wafer. The specific patterns are defined by masks on the wafer, and materials that are not protected by the masks are etched away by liquid chemicals. These masks are deposited and patterned on the wafers in a prior fabrication step using lithography. In this chapter, a silicon dioxide mask is used for silicon etching.

A wet etching process involves multiple chemical reactions that consume the original reactants and produce soluble products. It can be described by three basic steps: (1) diffusion of the liquid etchant to the structure that is to be removed; (2) the reaction between the liquid etchant and the material being etched away; in this case, the material is oxidized and then the oxidized material is dissolved; and (3) diffusion of the by-products in the reaction from the reacted surface.

Fig. 2 shows a silicon crystal (diamond-cubic) unit cell with arrangements of atoms and different planes. Liquid etchants etch crystalline (crystallographic etch) materials at different rates depending upon which crystal face is exposed to the etchant. In materials such as silicon, there is a large difference in the etch rate depending on the silicon crystalline plane, which can allow for very high anisotropy. Some of the anisotropic wet etching agents for silicon are potassium hydroxide (KOH), ethylenediamine pyrocatechol (EDP), or tetramethylammonium hydroxide (TMAH). Etching of (100) silicon wafers would result in a pyramid-shaped etch pit as shown in Fig. 3. The etched wall will be flat and angled. The angle to the surface of the wafer is 54.7°.

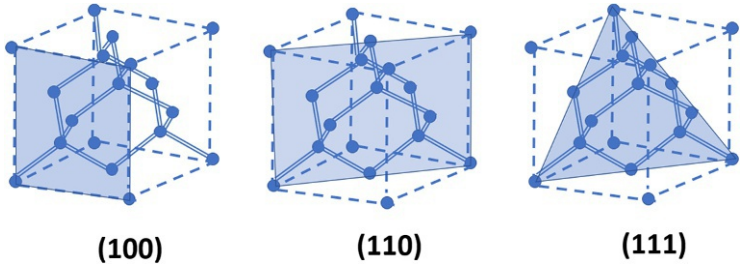

Fig. 2 Schematic of diamond-cubic unit cell showing various crystal planes.

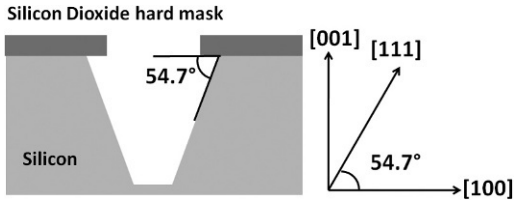

Fig. 3 Formation of facets on (100) planes of silicon.

In the following section, the standard wet chemical etching process used for fabrication of microneedle structures is reviewed. The advantage of this setup is its simplicity and low cost, which can be reproduced at any standard laboratory equipped with rudimentary microelectronic fabrication equipment.

3.1 Wet chemical etching process

A standard chemical bath with a sample mount, stirring mechanism, and temperature control was employed for this task. Fig. 4 shows the setup used for the various wet chemical processes. It should be noted that while the setup was quite simple—involving the mounting of the sample onto a clamp and positioning it inside the etchant liquid, which was stirred and kept at a constant temperature—it is critical to position the surface to be etched at an angle facing the bottom of the beaker. A number of runs were necessary to optimize this process and obtain a steady and repeatable etch rate. The dependence of the etch rate and surface finish on this angle is elaborated later in Section 3.3.

The etched pattern was observed using the stylus profilometer (Dektak XT), optical DIC microscope (Leica DM2700M), and scanning electron microscope (SEM) (ZEISS EVO18).

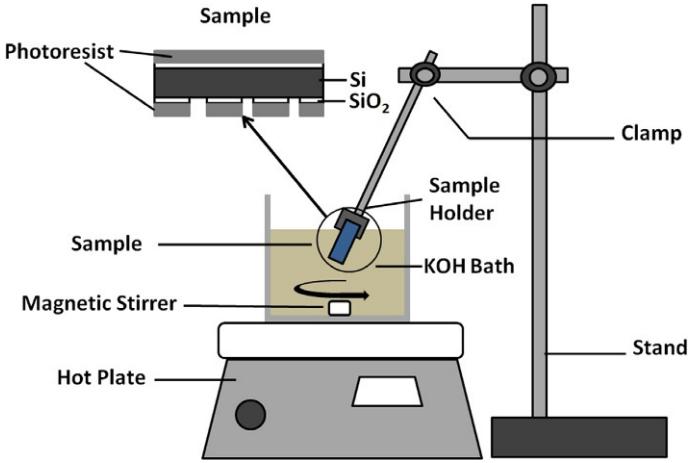

Fig. 4 Schematic of etching system for silicon micromachining.

3.2 Wet oxidation of silicon

Oxidation [22] was used at various stages for this work. For this oxidation, a tube furnace from Jay Crucibles was employed. Fig. 5 shows the complete oxidation setup for this purpose. The furnace is a two-stack two-zone system with a temperature-controlled zone of 10 cm long and a diameter of 2 in. Quartz tubes were used for this work, and the samples were placed on quartz boats and holders during the oxidation process.

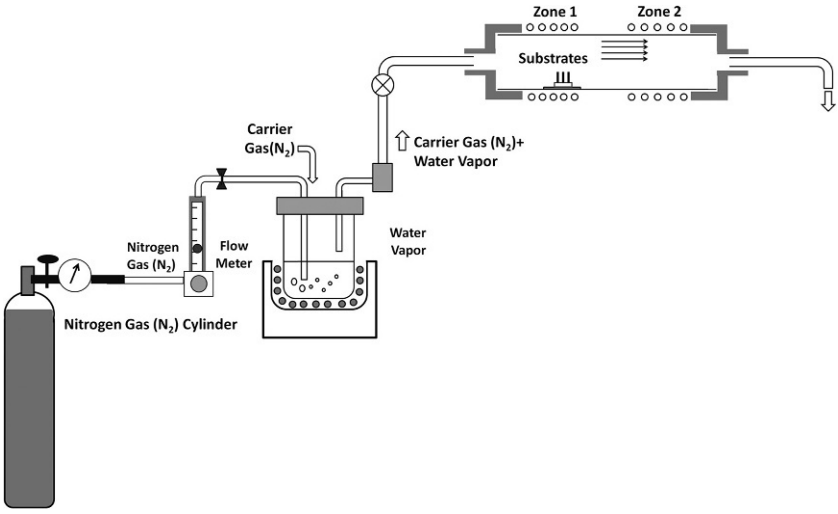

Fig. 5 Complete oxidation setup.

The system is capable of reaching 1100°C, even though significantly lower temperatures were employed for this work. Before operation, the uniformity of the temperature within the zone was calibrated using a retractable thermocouple.

Nitrogen was used as the carrier gas for the wet oxidation, and it was passed through a bubbler. Water vapor was generated and was carried by the nitrogen gas to the chamber of the furnace through the end caps. A substrate holder was placed at zone 1 of the furnace for wet oxidation.

The thickness of the oxide layer was determined by both ellipsometry measurements and the patterned etch process, where the etch height was measured using a stylus profilometer.

3.3 Wet chemical etching of silicon

Initial etching runs were carried out using a KOH (molarity = 3.28 mol/cc) solution. A solution of KOH was prepared using 35 g of KOH pellets (Merck) that was dissolved in 100 mL of DI water and heated to 80°C to form the chemical bath. The sample was introduced into the bath using the apparatus shown in Fig. 4. The solution was placed on a hot plate maintained at 80°C. The sample was maintained at a horizontal orientation. The process was carried out for a total of 7 h, but was interrupted every 2–3 h to measure the etch depth using a Dektak profilometer. From these measurements, as shown in Fig. 6, it was seen that the etch depth was increased linearly with time but the etch rate is very low (28 nm/min).

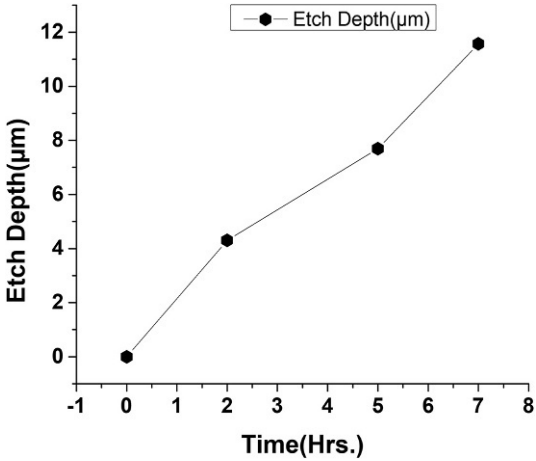

Fig. 6 Etch depth variation with time (before optimization).

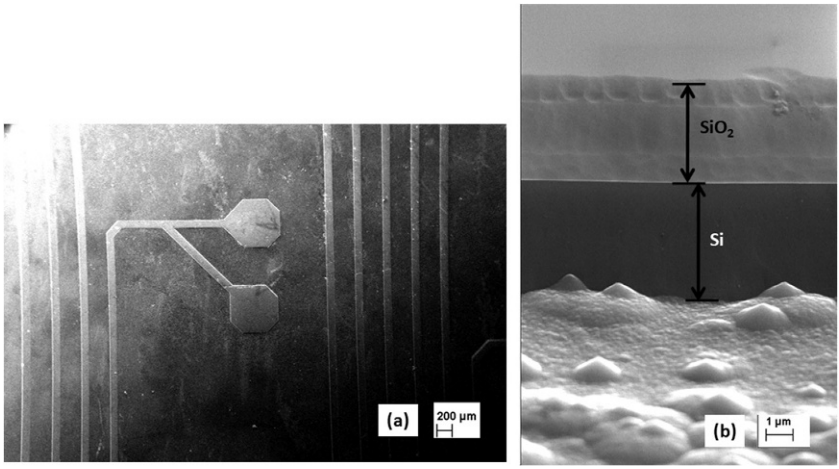

Fig. 7 Top view (A) and cross section (B) SEM image of ridge structures fabricated (before optimization).

The SEM image (Fig. 7A) shows the pattern that was etched into silicon by the KOH solution.

It is clear from this image that the edges were relatively straight and clear. The higher magnification image (Fig. 7B) taken at a tilted angle, however, shows that the etched surface was rough and contained rounded pyramidal structures.

After observing the previous etch results, modifications were made in the etch process. The same KOH molarity was maintained, but the temperature was increased to 120°C on the hot plate reading. The process was optimized based on the reading of the hot plate, but the solution temperature is expected to be significantly lower. The optimized etch rate is shown in Fig. 8.

The surface of the wafer was kept at an angle of 45° and it was facing downward at the time of the etching process, and the solution was stirred continuously. Critically, isopropyl alcohol (IPA) was added in the 1:5 volume ratio with KOH solution. After an etching of only 2.5 h, the silicon ridge structure was obtained, as shown in Fig. 9A.

SEM images of this ridge and the smooth sidewalls after the KOH etch are shown in Fig. 9.

The height of the silicon ridge was measured by a Dektak profilometer to be 122 μm. From the results, it appears that with the IPA the etch rate was increased and a rate of 814 nm/min was achieved. It has been reported that

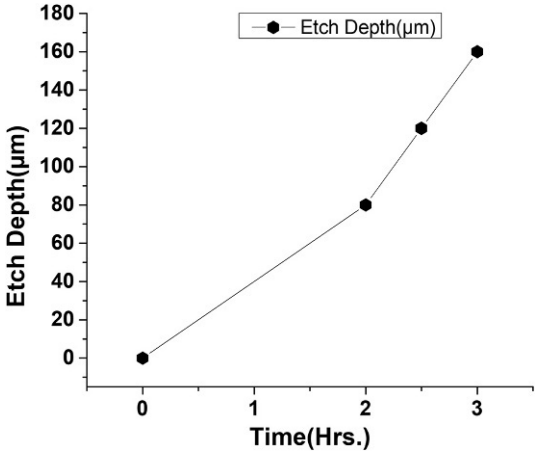

Fig. 8 Etch depth variation with time (after optimization).

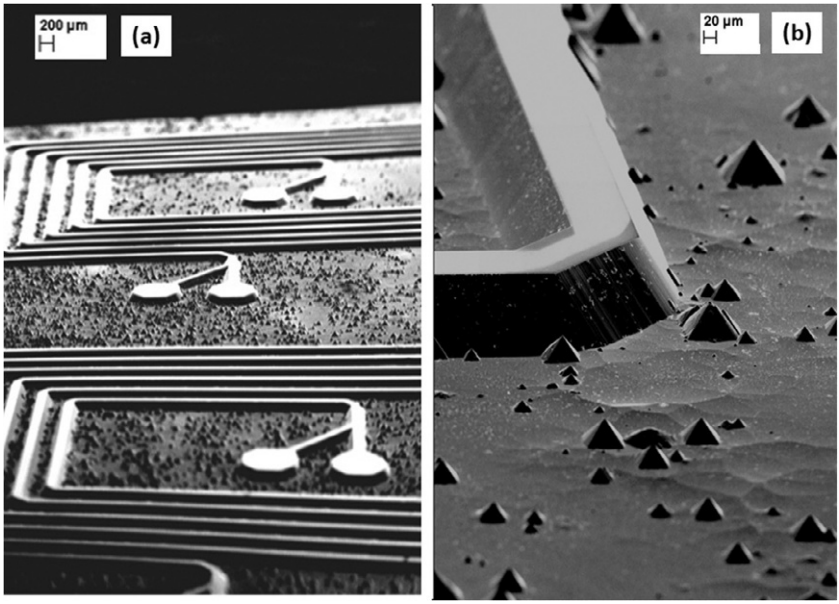

Fig. 9 Scanning electron microscope (SEM) image of the silicon mold (A) and the smooth and tilted sidewall of the ridge-like structure (B).

IPA molecules attach themselves to the (110) planes and inhibit the reactants from reaching the surface. This increases the ratio of the (100) etch rate to the (110) etch rate [23].

From Fig. 9B, it was clear that sharp sidewalls with striations indicating the crystal planes at the corners were achieved. The etched surface, while relatively flat, is decorated with small sharp pyramid-like structures (10–30 μm in height).

3.4 Effect of wet chemical etching on channel geometry

The results indicate that during the fabrication of the microchannel using crystallographic wet chemical etching there are many parameters that control the etching process and the shapes of the etched structures. The etch rate was enhanced by adding IPA to the KOH solution and increasing the temperature of the solution. It was also observed that moderate p-type doping of the silicon substrate helps to form the smooth side walls with 54.7° facets during anisotropic etching.

Two types of structures were used to study silicon undercutting under the silicon dioxide mask for edges that are rotated at various angles with respect to the [100] direction. Tapered rectangular hollow patterns, which covered 180 degrees of rotation, and solid rectangular patterns that covered 360 degrees of rotation were employed. FESEM images were obtained after the 8-h etch process, and the results are shown in Fig. 10.

The SEM images clearly show the etched openings made in silicon using the silicon dioxide mask. The undercut can be clearly observed in the SEM image (Fig. 10A) as a lighter border—indicating a free-standing SiO_2 layer—

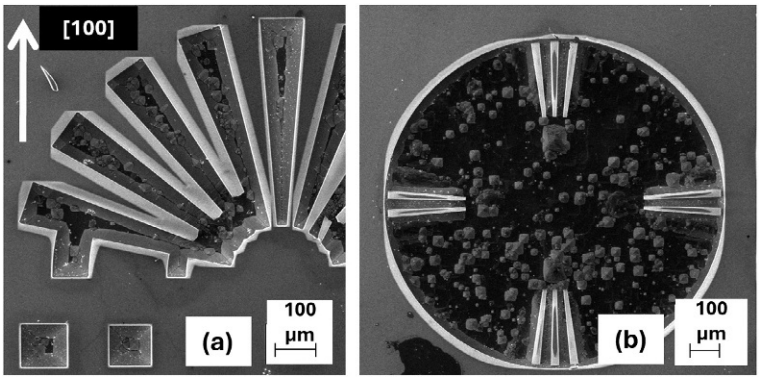

Fig. 10 Scanning electron microscopic images of (A) tapered rectangular hollow patterns and (B) solid patterns rotated w.r.t. the [100] direction.

surrounding the etched regions. The edges that are perfectly aligned with the [100] direction show minimum undercut under the SiO_2 mask layer, but the channels that are misoriented with respect to the [100] direction show progressively larger undercut as the angle of rotation increases. For very large angles, the SiO_2 layer is suspended freely forming a cantilevered tip.

From Fig. 10B, it is clearly observed that for high misorientation the top oxide layers were completely removed through undercutting during the process, which then allowed the silicon underneath to be etched. The silicon ridges that were still present after the etch process was completed were oriented close to the [100] and [010] directions.

Another very important observation was made from SEM imaging of the etched silicon ridge structures as shown in Fig. 11. Fig. 11A shows the sidewalls under high magnification, and the degree of smoothness as well as the slope observed clearly indicates both the advantages as well as the limitations of the crystallographic etch process. Fig. 11B shows the undercutting of the

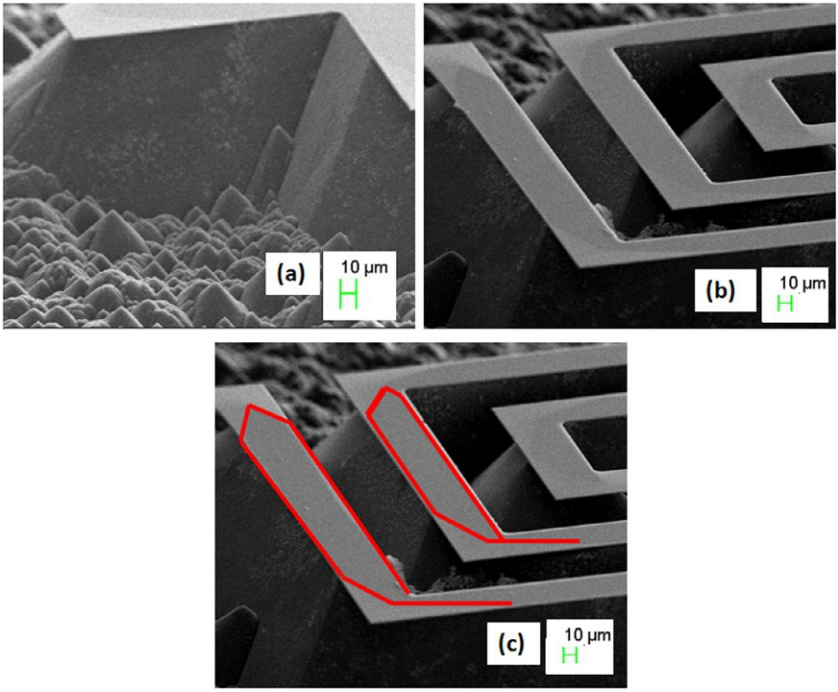

Fig. 11 SEM images of the (A) micromachined silicon sidewalls and (B) top view of silicon ridge and oxide layer. The actual geometry of the ridge structure formed by the undercut of the SiO_2 mask layer is shown in (C).

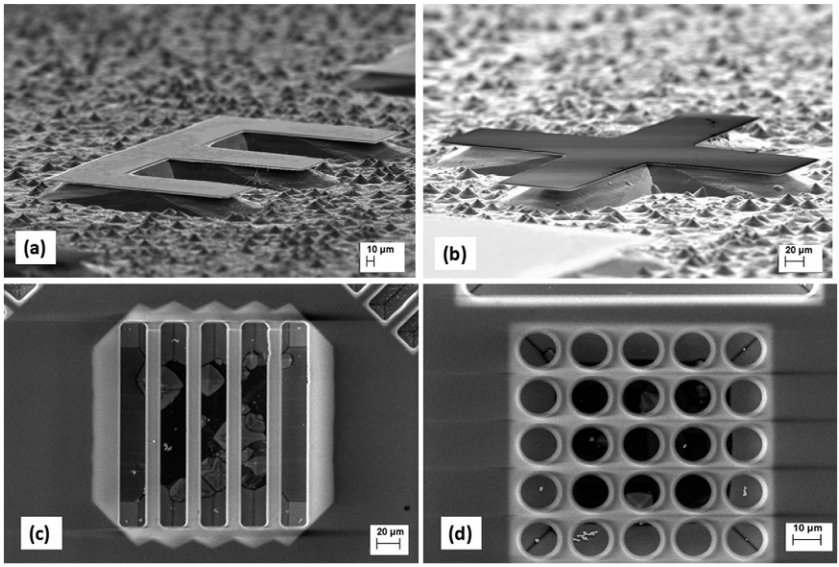

Fig. 12 SEM images of the various micromachined silicon structures with the SiO_2 mask layer (A)–(D).

silicon below the SiO_2 mask at the ridge corners. The darker region seen on top of the oxide layer is the image of the silicon surface below and is highlighted in Fig. 11C. The various sidewall facets are clearly observed, and two important conclusions can be drawn. Firstly, the etch process generates a large number of crystal plane facets at the corners. Secondly, the overall effect is that the "outer" corner (convex corner) is rounded by this process, while the inner corner (concave corner) remains true to the nature of the mask above. The overall effect is that at the corner of the etched "ridge" structure, the width of the ridge is different from that present at straight portions. Some other silicon micromachined structures are shown in Fig. 12A–D.

4 Microneedle fabrication processes

In the following, we describe the fabrication of microneedle structures with diameters of 10 µm to 30 µm and lengths up to 300 µm. Silicon substrates with (100) orientation were employed for this work. The thickness of the wafer was 500 µm.

Fig. 13A–E shows the process steps for the fabrication of micropyramidal arrays. The wet chemical etching process was employed for this purpose.

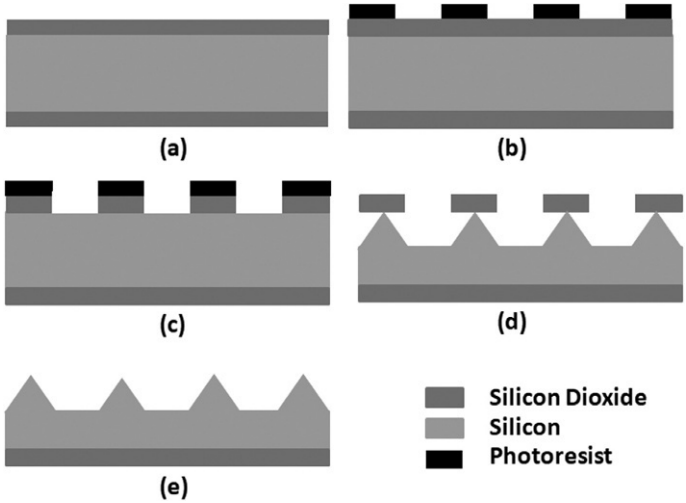

Fig. 13 Schematic of silicon microneedle fabrication steps (A–E) as described in text.

Box-like patterns were used as a mask for the photolithography. The sides were ranging from 80 μm × 80 μm to 140 μm × 140 μm with different spacing. These square features were formed on the SiO_2 surface using the photolithography process. Type A (p-type silicon) and type B (n-type silicon) substrates were employed for microneedle array fabrication. Photoresist S1813 was spun on the substrate at 3500 RPM for 35 s, followed by prebake at 115°C for 1 min. Care was taken to align (using mask aligner MA6/BA6) the sides of the square patterns with the [100] direction of the wafer. This alignment was necessary for the subsequent crystallographic etch.

The sample was exposed to UV light in the soft contact mode for 4 s. After the exposure, the sample was developed using MF26A till a clear pattern was observed. The whole procedure was performed in a Class 100 clean room environment. This area has been certified to contain a maximum of 100 numbers of 0.5-μm-diameter dust particles in one cubic foot of air.

The buffer oxide etch (BOE) solution at room temperature was employed for the selective etching of the SiO_2 layer, and the BOE solution had an etch rate of ~50 nm/min (Fig. 13C). The backside of the wafer was covered with Kapton (Polyimide) tape during the BOE etching process to protect the silicon oxide. After that, the photoresist was removed and the wafer was employed for the crystallographic etch of the silicon (shown schematically in Fig. 13D). Square SiO_2 regions, formed after step-c, were used as the hard mask for the crystallographic wet chemical etching. A solution of

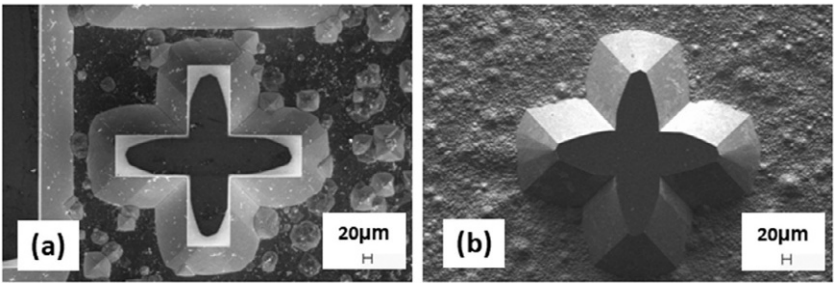

Fig. 14 Etched structures on (A) type A and (B) type B substrates as described in text.

KOH was prepared using 35 g of KOH pellets (Merck) that was dissolved in 100 mL of DI water and heated to 80°C to form the chemical bath. The sample was introduced into the bath using the apparatus shown in Fig. 4. An etch rate of up to ~540 nm/min was achieved, under optimized conditions.

A comparative study was carried out when the same etching process was performed for two samples, of type A (p-type silicon) and type B (n-type silicon). The SEM images obtained after the entire process steps for the two samples are shown in Fig. 14A and B, respectively. It is observed that the final etched silicon structures were nearly similar for the two samples. However, while the SiO_2 layer was intact for the p-type sample, it was completely removed for the n-type sample at the end of the process step. It is believed that for the n-type sample there is a presence of phosphorus in the oxide layer, due to diffusion from the silicon itself. This makes the oxide layer porous and brittle. However, the KOH etching of silicon was the same for both samples and they showed similar clean facets.

For the KOH etch process if IPA was not added to the etching solution, multiple silicon facets were observed. Therefore IPA was added to the KOH solution in 1:5 volume ratios. IPA not only increases the etch rate, but it also helps for smoothening the silicon sidewalls during crystallographic etch.

In this fabrication process, a high degree of control is necessary to get a narrow tip. It is critical that the oxide mask (hard mask) remains on the top during the silicon crystallographic etch. During this process, the undercut occurs below the SiO_2 mask, thereby generating the facets of the microneedle. After the complete undercutting process, the tip is automatically removed. It is necessary to stop the etching process before its complete removal. Fig. 15A shows the microneedles with still attached oxide caps. If the etching process is not stopped, the unprotected tips become blunt as they get rounded off. Fig. 15B shows the round-top microneedles formed by an overetching process.

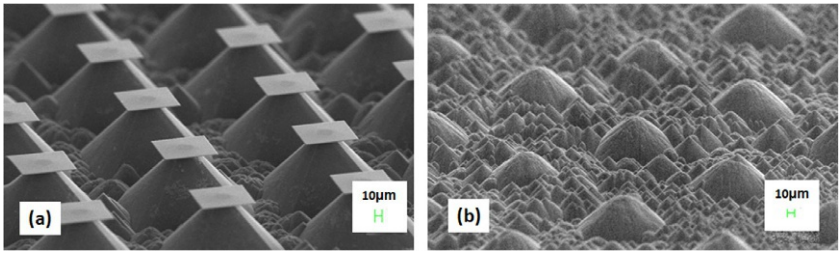

Fig. 15 Effect of (A) underetching and (B) overetching during microneedle fabrication.

5 Microneedles with tip diameter reduction

In the effort to generate the narrow microneedle tip, a two-step process was taken. The process steps were the following: (a) truncated top micropyramid formation and (b) narrowing the tip using wet chemical etch. This two-step approach will increase the stability of the fabrication process. The dependency of thermal oxidation of silicon (on surface morphology) was employed in the second process step. It was observed that the sharp edges of silicon are oxidized faster than the flat surfaces [24]. At the initial step of that two-step process, the crystallographic etch process was stopped at that time when a small flat plateau remained on the top. On these micropyramidal structures, wet oxidation was performed in a tube furnace at 950°C for 10 h. The thickness of the oxide layer grown on an unetched silicon wafer was measured by spectroscopic ellipsometry, and the thickness was about 870 nm. It is shown schematically in Fig. 16 that the oxidation process will

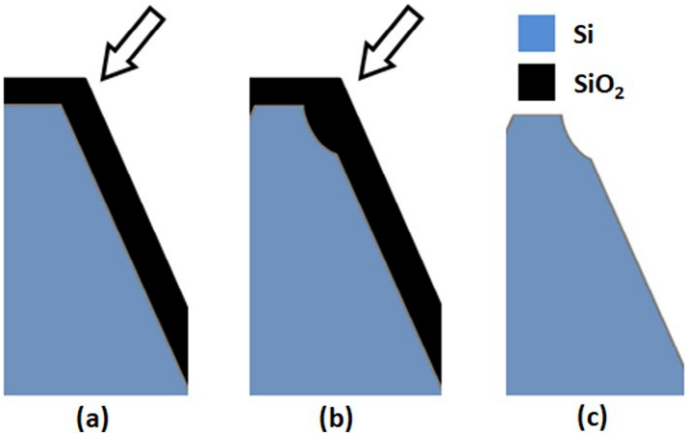

Fig. 16 Schematic of process steps (A–C) for the reduction of tip diameter (as described in text).

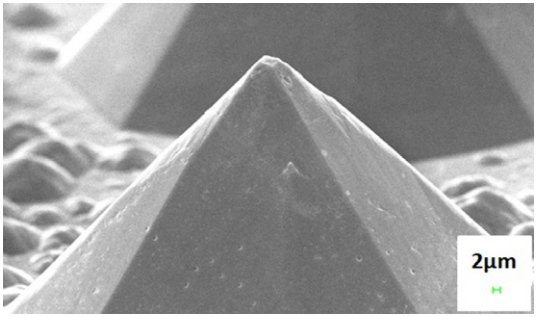

Fig. 17 Silicon microneedles with reduced tip diameter.

be slower on flat sidewalls and the top plateaus of the silicon micropyramids (Fig. 16A) and will be faster at the corner of the plateaus (Fig. 16B).

SiO_2 from all regions was removed by a subsequent BOE (Fig. 16C). In the SEM image shown in Fig. 17, this can be observed clearly. In this process, it is clearly seen that the top flat plateau has been reduced. The dimension of the top plateau was a square of less than 2 μm. The tip size of these microneedles is acceptable for biomedical applications. The advantage of this process is there will be no need for precise process termination at the time of the crystallographic wet chemical etch process. So larger or a smaller top plateau can be generated by the modification of the time and temperature of the oxidation process. To develop sharp microneedle structures, several groups have attempted the isotropic etching process and they have successfully generated a significantly narrower tip. But for this process silicon nitride mask is required [19] and for this purpose chemical vapor deposition (CVD) process is necessary. But CVD is more complex than the thermal oxidation process that is used in this work.

6 Microneedles with tip modification: Incorporation of spontaneously formed microcavities for fluid transport

The effect of the HNA etch process has been investigated on the truncated micropyramidal structures that were fabricated by anisotropic KOH etching. The formation of pyramidal structures was described in Section 4, and thermal oxidation on such structures was described in Section 5. When these structures were dipped in a BOE bath (at room temperature for 40 min), all the SiO_2 that includes the top cap layer of the micropyramids was removed. Now a bath of (48%) HF:concentrated HNO3:glacial acetic acid in a ratio of 4:7:11 (HNA) was employed at room temperature. In this bath, the samples were immersed maintaining an angle of 45°; they were faced

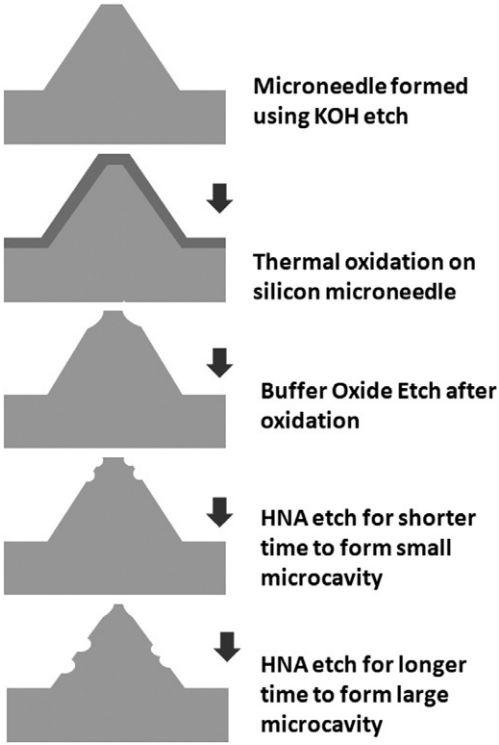

Fig. 18 Schematic of process steps for the incorporation of microcavities.

downward for 5 min. Two sets of samples were fabricated for this purpose. For the first set, the solution was stirred at 120RPM. For the second set, the solution was kept stagnant. The microcavity formation on the tip of the microneedle is shown schematically in Fig. 18.

Scanning electron microscopy was performed after rinsing and drying the samples. The SEM image of the microneedle array structures is shown in Fig. 19. These are the arrays used for the first HNA process where stirring was performed during the process. The significantly narrower array is seen, and there was no rounding off at the edges. The octagonal tip (diameter: 25–30 μm) became square tip (diameter: 10 μm), so the tip was also reduced. The sidewalls were also changed. Previously there was interlinked (311) and (111) planes, but now more regular structures are seen bound by only the (111) planes.

For the second HNA process where the solution was not stirred, an interesting phenomenon was seen. There were no smooth sidewalls for these samples; rather microcavity structures on the sidewalls were found. It is

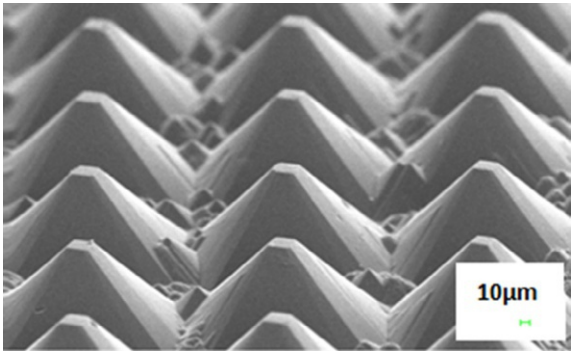

Fig. 19 SEM image of the narrow tip micropyramidal array.

observed in the SEM images that large microcavities (about 20–30 µm in diameter) are produced on the sidewalls of micropyramidal structures (Fig. 20A) for longer HNA time. The density of the microcavities may depend on different parameters like array geometries.

For short HNA etch times, microneedles were found with a large number of microcavity structures at the tip edge. The size of the microcavities was varying from 4 µm to 10 µm in diameter (Fig. 20B). Thus, the combination of these three processes, (a) crystallographic KOH etch, (b) thermal oxidation, and (c) HNA etching process, can generate micropyramids with microcavities on the tip. This type of structure can be used to store drugs during the drug delivery process through the human skin. This process is not only simple but a low-cost process also, and the structures are very robust as the micropyramids are surrounded by (111) crystal planes. The tip of such structures is not totally porous, as a result the possibility of breakage at the time of drug insertion is less.

There are two possible phenomena that can link the formation of such microcavities. During the thermal oxidation process, the rates of thermal oxidation are different for different surfaces. A flat surface has a uniform oxidation rate, but for the sharper edges that are at the top of the micropyramid, oxidation rate can differ widely. Thus LOCOS type structures are formed, and during the etching process, these are etched away. There may be another reason for the formation of such cavities. There were small fluctuations of chemical composition in the HNA solution especially at the tip of the pyramids. The defects were present at the meeting points of the facets, which were generated earlier KOH etch. The localized etch rate at those points may be quite high. The generation of microcavities is due to the faster removal of these localized defects.

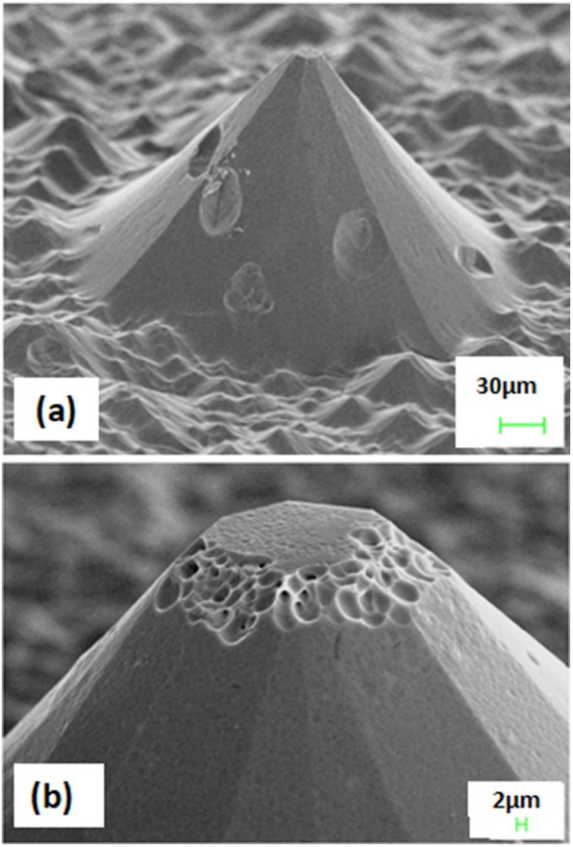

Fig. 20 SEM images of (A) microcavities on the sidewalls of the micropyramid and (B) microcavity ring surrounding the tip of the micropyramid.

This HNA process can be optimized to any degree of surface porosity by changing the etch process time (longer or shorter); there will be no need for stirring.

7 Fluid storage in microcavities

The optical microscopy was carried out using a LEICA DM2700M system to verify whether the series of microcavities at the edge of the top plateau of the microneedles are capable to store a fluid within the cavity or not. Rhodamine B dye was used to coat the microneedles. The visible light and a handheld UV source with excitation at 365 nm were employed to illuminate the microneedles.

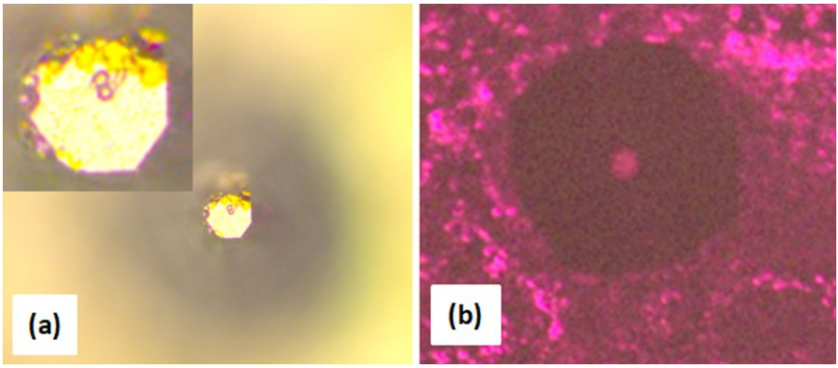

Fig. 21 Visible light microscopy image (A) and fluorescence image (B) of a microneedle with microcavities filled with rhodamine B dye. Inset in (A) shows a magnified view of the microneedle tip.

Fig. 21A shows a visible light microscopy image of a microneedle with the tip. The inset image is at higher magnification, and it indicates that the plateau is octagonal (30 μm in diameter). It is observed from the images that the rhodamine B dye is there in droplet form (about 6–7 μm in diameter) at the edge as well as at the top of the plateau. Therefore, microdroplets are observed within chemically etched microcavities, so the fluid can be stored within the microcavities.

The fluorescence of the rhodamine B dye can be seen by illuminating UV light on the structure. The fluorescence from the dye can be seen clearly in Fig. 21B. Fluorescence is seen from the microcavity only; there was no fluorescence from the smooth walls of the microneedle. Therefore this kind of structure can be a good choice for storing the drug in the microcavity where fluid can be selectively stored. As a result it will reduce the consumption of expensive and dangerous drugs at the time of the injection process.

The use of tomato skin as a model transdermal skin mimic has been reported by other groups [25]. Similar tests were carried out and the results are presented below.

The microneedle array was used to press against the tomato skin that was maintained at room temperature. After this insertion process, the microneedle array was withdrawn and the tomato surface was placed under a microscope. As shown in the inset in Fig. 22, the impressions made against the tomato skin were clearly observable. In higher magnification, the puncture mark made by the microneedles, as well as the vegetable fluid exuding from it, was clearly viewable.

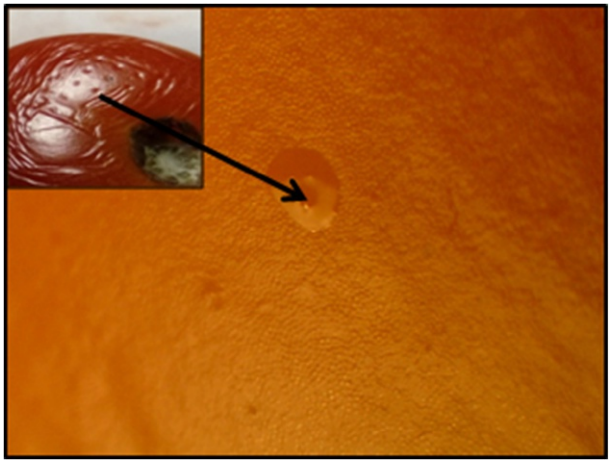

Fig. 22 Microscope image of tomato skin pierced using a microneedle. Inset shows the impression of an entire array.

These results give an indirect indication of the use of microneedles in skin penetration. No damage was observed on the microneedle structures after repeated insertions, indicating their structural stability.

8 Hollow microneedles

In this section, the design and simulation of hollow microneedles and technology for the fabrication of the same is discussed.

COMSOL Multiphysics was used for the simulation of fluid flow through a poke-and-release microneedle. In addition, the stress applied to the microneedle during introduction into the skin has been also simulated. Here the height of the microneedle is 150 μm, and the designed structure is shown in Fig. 23. There is an inverted pyramidal cavity at the back of the wafer that forms the reservoir of the fluid to be injected or withdrawn. This is aligned with a pyramidal needle structure on the top surface as shown in the figure, which will penetrate the skin. Both of these structures are bound by the (111) planes of silicon and can be formed by crystallographic etching. The connection between the reservoir and the needle is through a narrow channel 20 μm wide, which can be formed by deep reactive ion etching (DRIE).

The maximum stress generated in the microneedle when 3 MPa pressure is applied to the microneedle, which is needed to push a needle into the human body [26], was simulated. Fig. 23B shows the top view, and it can be seen that the stress was generated mostly at the base of the

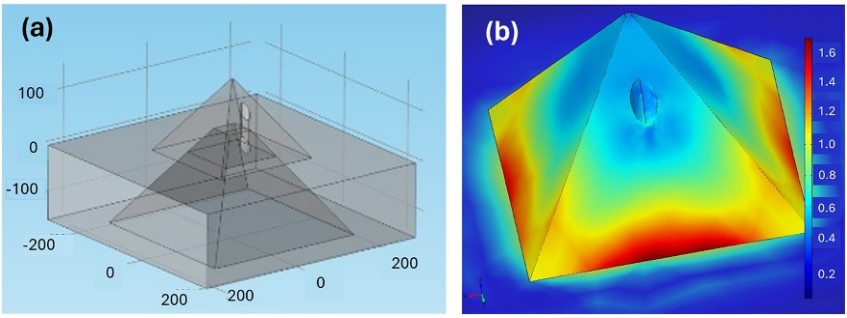

Fig. 23 (A) Hollow microneedle structure and (B) simulated structure showing minimum buckling.

microneedle and to a weaker degree just around the nozzle of the needle. Other studies indicate that the structure is robust with minimal buckling during penetration.

The flow through the nozzle was also calculated for different inlet pressures ranging from 10 kPa to 100 kPa and for different radii. The pressure dependence on liquid velocity is given in Fig. 24 for three different nozzle radii maintaining a possible reasonable flow rate of fluid through the channel based on the Navier-Stokes equation.

$$\frac{\partial \boldsymbol{u}}{\partial t} + \boldsymbol{u}.\nabla \boldsymbol{u} = -\frac{\nabla \mathrm{p}}{\rho} + \nu \nabla^2 \mathbf{u} \tag{1}$$

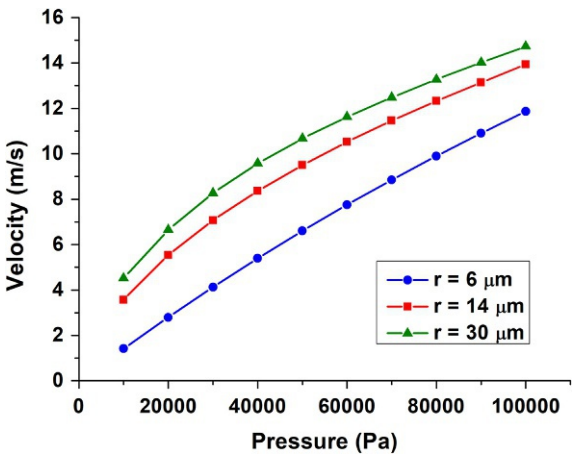

Fig. 24 Flow rates for different nozzle diameter.

where u is the fluid velocity vector, P is the fluid pressure, ρ is the fluid density, υ is the kinematic viscosity, and ∇^2 is the Laplacian operator.

Fig. 25 shows the schematic of the fabrication steps for these structures that consist of a nearly pyramidal microneedle as described previously in Section 4, which has been modified to incorporate a vertical tube of 20 μm in diameter for injection into the body.

The steps are (i) initial formation of the vertical tube using deep reactive ion etching (DRIE), (ii) a front surface lithography and selective removal of the oxide layer, (iii) a back-aligned lithography process to remove partially the back oxide layer, and (iv) crystallographic wet chemical etch process to fabricate the top microneedle and bottom reservoir structure simultaneously.

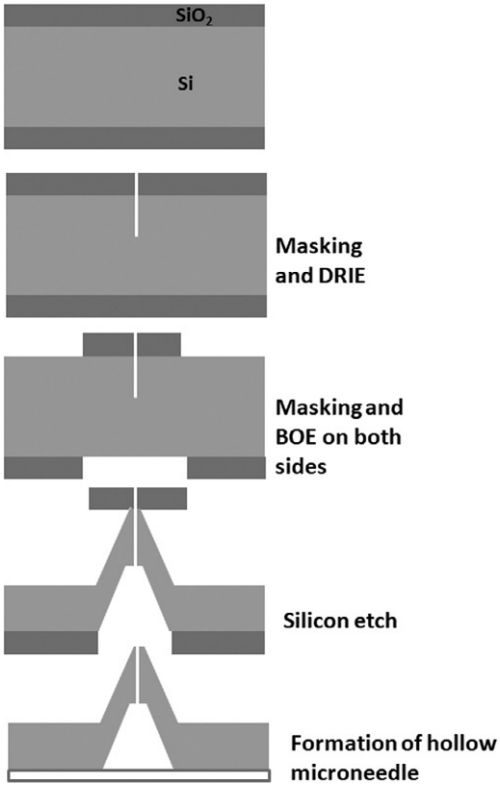

Fig. 25 Fabrication steps for hollow microneedle.

9 Conclusions

In this chapter, we review the fabrication technologies employed in the development of microneedle structures in general, as well as our recent results on the fabrication of silicon-based microneedles using simple techniques such as crystallographic wet chemical etching. The optimization process steps are discussed in detail, and the development of both microneedles with reduced tip diameter as well as microneedle structures with incorporated microcavities for efficient drug loading has been presented. Finally, simulation studies as well as the technology pathway for the fabrication of hollow microneedles using DRIE and other techniques have been mentioned briefly.

The development of microneedles based on silicon is essentially a small part in the larger developmental pathway to the realization of silicon-based lab-on-chip systems, where all components, microfluidic, electronic, and mechanical, may be integrated. If low-cost techniques are employed to create the body-fluid test structure, which is not reusable for obvious reasons, the LOC can be deployed on a very large scale and helps bring affordable health care to a much wider range of the population.

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CHAPTER 9

Microneedle for wound healing and dermal application

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1 Introduction

1.1 Wound

Injuries that cause the skin or other body tissues to become lacerated are called wounds. They include skin that has been sliced, scraped, scratched, or pierced. Wounds are often the result of an accident; however, they may also result from surgery, sutures, or stitches [1]. Burns, surgeries, and other types of traumas are the most common causes of dermal injuries [2]. During the healing process, scars often form as a consequence of these injuries, which affects the normal skin's ability to function normally and may also cause psychological and aesthetic harm [3]. Numerous therapeutic interventions and nonsurgical methods, such as silicon dressings, radiation, laser therapy, and radiotherapy, have been used for scarless repair [4,5].

1.2 Wound healing

The wound healing process in the skin is dynamic and intricate, requiring a delicate equilibrium between various cells, cytokines, growth factors, pathways, and the production of matrix (ECM) [6,7]. Scarring is accelerated by the cytokine's interleukin-6 and IL-8, which are considered to be pro-inflammatory; however, the antiinflammatory cytokine IL-10 has the opposite effect [8–10]. Less scar tissue has developed due to the simultaneous addition of several growth factors [11–13], cytokines, and cells to wound matrices, but the problem of scarless outcomes has not yet been resolved [14]. As the healing process progresses through remodeling phases, inflammatory responses and angiogenesis undergo dynamic alterations at various stages to affect the development of the ECM and, eventually, scar formation [15,16]. The complex variables (growth factors, cytokines, cells, and ECM)

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control angiogenesis and inflammation at different phases of the healing process to determine the outcomes of scars [17]. Various biomaterials and cell-laden matrices have been developed to promote the healing of scarless wounds and actively control angiogenesis and inflammation. These matrices typically generate suitable microenvironments for wound healing [18].

2 Wound healing process

The dynamic process of wound healing is a fundamental physiological event that involves the supportive effort of many different cells (platelets, fibroblasts, macrophages, and so on) and the products produced by the cells. When we suffer a skin wound, our bodies set in motion a chain of reactions known as the "cascade of healing" [19]. The four phases of wound healing are shown in Fig. 1.

3 Transdermal drug delivery

There has been a recent uptick in interest in transdermal delivery as a potential method of drug administration. The drug is able to enter systemic circulation through the skin without any loss of the drug while it is in the process of reaching its target. This results in an increase in bioavailability, an improvement in sustained drug release, a reduction in unwanted side effects, and an improvement in the physiological and pharmacological response [20,21]. For example, transdermal delivery solves the problems associated with oral and intramuscular delivery of testosterone, bypassing the hepatic first-pass following oral treatment and reducing the obligatory dosage. Furthermore, it removes the need for recurring injections, resulting in a more significant concentration of testosterone in the blood [22,23].

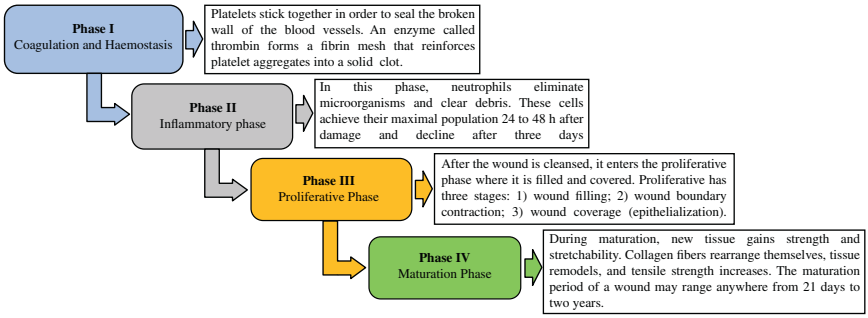

Fig. 1 Phases of wound-healing process.

Nonetheless, drug distribution through the transdermal administration route is heavily influenced by drug chemical characteristics, which alter absorption via the subcutaneous (SC). As a result, few medicines can be administered in significant therapeutic levels using transdermal drug delivery [24].

4 Microneedles (MNs) for drug delivery

Microneedling is the procedure of repeatedly puncturing the skin with sterilized microneedles to stimulate collagen production. It was first proposed in 1995 by Orentreich and Orentreich, who used the term "subcision" to describe using hypodermic needles to promote wound healing in depressed cutaneous scars [25]. The first MN product, the original Dermaroller, was created by Dr. Desmond Fernandes in 2006 [26]. MN is a relatively inexpensive and slightly invasive way to treat several cosmetic and skin conditions [27].

As shown in Fig. 2, there are five distinct types of MNs based on their construction: solid MNs, hollow MNs, coated MNs, dissolving MNs, and hydrogel-forming MNs. The "poke and patch" method of solid MN distribution involves first employing MN arrays to create holes in the skin and then using a transdermal drug patch to distribute a conventional drug formulation [28,29]. Coated MNs are applied to the skin by the "coat and

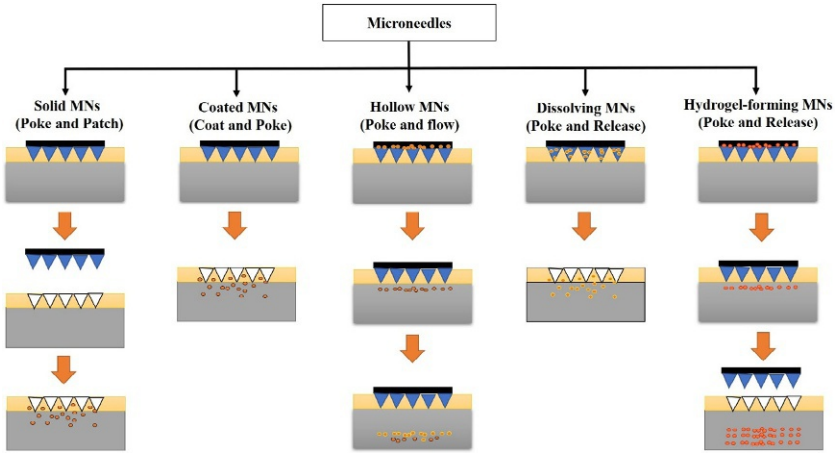

Fig. 2 The mechanism of drug delivery of solid microneedle (solid MN), coated microneedle (coated MN), hollow microneedle (hollow MN), dissolving microneedle (dissolving MN), and hydrogel-forming microneedle (hydrogel-forming MN).

patch" method, in which a drug formulation is coated onto the microneedles. Once the coating dissolves upon skin penetration, the drugs are deposited into the skin. The drug is loaded into the hollow tip of the microneedle and then deposited into the epidermis or higher dermis layer of skin upon insertion, called the hollow microneedles method. It is known as "poke and flow" in its basic form [30]. In contrast to hydrogel-forming microneedles, which absorb huge amounts of water into their polymeric network and expand, the dissolving microneedles are often created from nonbiodegradable polymers and need simple steps for application [24,31]. Poke and release is the name given to the technique of dissolving and hydrogel-forming MN drug administration; both procedures circumvent the need to take specific precautions for discarding the needle and the possibility of unintended reuse of the MN [32,33].

4.1 Type of microneedles

4.1.1 Solid microneedles

The "poke and patch" method involves using solid MN to perforate the skin, producing microchannels that penetrate the deepest layers of the epidermis. Because the stratum corneum, the primary barrier to permeability, is broken down by this approach, the passive transport of medicines through the skin is considerably improved [34,35]. The following are the two stages involved in this method: First, the MN are used to penetrate the epidermis, and they are then removed. Second, the medicine is given in a standard dose form (solution, cream, or patch) as an external drug reservoir [36].

4.1.2 Coated microneedles

The "coat and poke" method is another method that may be used with solid MN. This method involves covering the surface of the solid microneedles with a medication or vaccine-loaded formulation before inserting them into the patient [37]. Following the insertion of the MN, this method permits drug diffusion from the surface of the coating into the deeper epidermal layers [38] (Fig. 2.). The utility of this method is severely restricted due to several factors, most of which are associated with the coating. For example, the quantity of medicine that can be contained in the coating layer surrounding it is not very high. In addition, the thickness of the coating has the potential to lessen the sharpness of the microneedles, which in turn may affect their capacity to puncture the skin [39].

4.1.3 Dissolving and hydrogel-forming microneedles

A wide variety of water-soluble and biodegradable materials may be used to make dissolving MN. These materials allow the medications to be placed into the MN and then released when the MN dissolves [40,41]. This method advances the "poke and patch" technique because it allows regulated drug release to be maintained over a more extended period using dissolving microneedles. The release is accomplished by managing the pace at which the formulation used as the MN matrix dissolves. MN can puncture the skin and remain implanted until full breakdown, another benefit of this method. It allows the process of administering the medication to be condensed down to a single step. In addition, dissolving MN prevents the production of sharps waste, reducing the expenses associated with its treatment and the risk of accidents caused by needles [42,43]. On the other hand, some disadvantages include a reduced medication loading capacity and a possibly diminished capacity to perforate the *stratum corneum*.

Hydrogel-forming MN or swellable MN are two ways that have been developed as an alternative to "poke and patch" techniques. After being inserted into the skin, these devices are intended to draw in the skin's interstitial fluid to create uninterrupted and continuous microchannels among the dermal capillaries. This method enables the release of medications with a lower therapeutic index stored in a drug reservoir as an attached patch [44,45].

4.1.4 Hollow microneedles

The "poke and flow" method was devised to deliver the drug solution into the skin in a manner that mimics hypodermic injections while overcoming the constraints of these injections. After the skin has been perforated, the microneedles acquire a function analogous to that of hypodermic needles in that they are used to administer drug formulation. The production of hollow microneedles is complex and costly because of their micrometric size, requiring significant technical resources [46,47].

4.2 Microneedle for wound healing

During the healing process, excess fluids are produced, which strongly correlate with how well the wound is healing. Several studies are now looking into how to make gadgets that can simultaneously observe the wound surroundings and help to heal it. Gao et al. reported that a double-sided integrated microneedle array (MA) (i-SMD) can not only monitor the physiology of wounds but also release drugs based on the surroundings of the

epidermis to get the best healing effect [48]. Ziesmer et al. administered vancomycin (VAN) using MNs through a topical route. The MNs can penetrate the dermal barriers of both porcine and human epidermis. According to permeation experiments conducted on the porcine epidermis, the preponderance of the administered VAN is retained within the skin. It is demonstrated that the VAN-MN array inhibits methicillin-resistant *Staphylococcus aureus* (MRSA) proliferation in vitro and on epidermis ex vivo. The VAN-MN arrays can be applied to multiple medications and may facilitate the localized treatment of MRSA-caused cutaneous infections while minimizing adverse systemic effects [49]. Yin et al. developed a magnesium organic framework-based microneedle patch (MN-MOF-GO-Ag) that accomplished transdermal delivery and combination therapy for diabetic wound healing. The full-thickness cutaneous wounds of a diabetic mouse model are used to illustrate the therapeutic benefits of MN-MOF-GO-Ag on wound healing. Treating mice with MN-MOF-GO-Ag results in a considerable improvement in the wound healing rate [50]. Guo et al. developed a glucose-responsive insulin-releasing hydrogel for microneedle dressing. The resulting hydrogel microneedle dressing was superior to hydrogels without microstructures in terms of adherence to the skin, glucose-responsive insulin release behavior, and mechanical characteristics. Reduced inflammation, increased collagen deposition at regeneration sites, and better blood glucose control in animal models suggest microneedle dressings may hasten the repair of diabetic wounds. Thus, the glucose-responsive insulin-releasing hydrogel microneedle dressing helps manage diabetic wounds and may be useful in addressing other chronic skin injuries [51].

To promote scarless wound healing, Jinying et al. created a transdermal drug delivery system using BSP/chitosan (CS) composite bilayer dissolvable microneedles (CS-AS-BSP MNs) containing asiaticoside (AS). The effect of CS-AS-BSP MNs on healing wounds was tested in an experiment with a full-thickness cut on a rat. After 3 weeks, the wound had fully healed without any scars. Also, CS-AS-BSP MNs turned down TGF-1 and COL I, which are fibrosis-related genes, to inhibit scarring [52]. Qi Lei et al. hyaluronic acid (HA)-based MNs to promote skin tissue regeneration. The prepared microneedle shows excellent wound-healing properties [53]. Junjie et al. developed a chitosan microneedle array (CSMNA) to promote wound healing. Chitosan is biodegradable and biocompatible [54], and it also helps in wound healing [7]. It has been shown that the biomass CSMNA patch facilitates wound healing by reducing inflammation, increasing collagen

deposition, promoting angiogenesis, and regenerating damaged tissues. Because of its adaptability, this CSMNA patch may be used in clinical settings for wound management [55]. Ayesha et al. used chitosan and fucoidan to prepare moxifloxacin (MOX)-loaded nanoparticles (MOXNPs) incorporated in a pullulan-based microneedle patch. To accomplish fast hemostasis/analgesia and persistent bactericidal activity, the author of this work uses a combination of polysaccharides to create a smart multifunctional microneedle platform [56]. Yang et al. prepared hyaluronic acid-based microneedle patch to accelerate diabetic wound healing. In vivo studies on mice with diabetes induced by streptozotocin indicate that the prepared microneedle accelerates wound healing without causing systemic toxicity [57]. Park et al. prepared to evaluate the wound-healing potential of microneedle composed of hyaluronic acid and green tea (GT) extract. The GT/HA microneedles were shown to be antimicrobially effective by reducing the growth of both gram-negative (*Escherichia coli*, *Pseudomonas putida*, and *Salmonella typhimurium*) and gram-positive (*S. aureus*, and *Bacillus subtilis*) bacteria by at least 95% [58]. Asad et al. prepared MN using pH-sensitive polymers for wound-healing applications. The finding reveals that the prepared formulation demonstrated wound pH-sensitive drug release in vivo (using rats) as well as in vitro (using phosphate-buffered saline and porcine skin) with quick response. At a pH that is considered to be optimal for healthy skin, which is 4.5, a negligible amount of MNs was found to be released into the test medium. However, when MNs were subjected to wound pH conditions (pH7.5), increase in the drug release rate was observed [59].

4.3 Microneedle for insulin delivery

Insulin is perhaps the most thoroughly researched hormone to be administered through a route other than the SC. Resnik et al. developed and produced hollow silicone MN for insulin microinjection. In vivo findings revealed an effective infusion of fast-acting insulin as measured by monitoring plasma glucose levels. Microneedle-based administration, in particular, resulted in a less substantial decline in glucose levels but a significant rise in serum insulin levels (40–50% increase) owing to more effective exogenous insulin delivery [60]. Luo et al. formulated a microneedle array for glucose-response insulin delivery. The pH-sensitive insulin-loaded nanoparticle with glucose catalase and oxidase-containing pH-insensitive nanoparticles loaded microneedle patch was developed in this research work. The in vivo investigation conducted on mice with type-I diabetes shows that

the platform can efficiently manage blood glucose levels within normal limits for an extended period [61]. Zhao et al. prepared soluble microneedles by using polyvinyl alcohol (PVA) polyvinylpyrrolidone (PVP) for transdermal delivery of insulin. The in vivo finding reveals that the prepared microneedles are a potential tool for transdermal drug delivery [62]. Chen et al., who synthesized MN using poly-c-glutamic acid (c-PGA) and polyvinyl alcohol PVP as supporting structures, designed it to dissolve in the skin in 4 min. The hypoglycemic effect attained with insulin-loaded MN was equivalent to that seen with a standard SC insulin injection, as evidenced by the findings obtained in a model using rats. The bioavailability of insulin was approximately 90%–97%. In addition, there were no discernible variations seen in the plasmatic insulin concentration profiles after several administrations, which is evidence of the reliability and precision of the MN [63]. Lee et al. used a two-step casting and spinning process to prepare to dissolve MN using gelatin and carboxymethyl cellulose. It was done to concentrate the insulin in the needle tip and make cutaneous administration more effective. Ex vivo results showed that after 1 h, 50% of the insulin was released and penetrated the skin. After 5 h, the total amount of insulin penetrated through the skin was 80% of the initial dose. In vivo, tests showed that 95.6% and 85.7% of the insulin in MN were bioavailable [64].

Courtenay et al. revealed that the permeability of Bevacizumab was significantly improved by using MN, with the hydrogel-forming MN allowing for the absorption of roughly 25% of the available antibody. In comparison, the control solution without microneedles only achieved 0.9%. Controlled antibody delivery was further validated by in vivo tests, which showed that the medication remained in the plasma for 7 days after a single administration [65]. In another study, Yu et al. developed nanovesicles encapsulating MN and loaded them with insulin and glucose oxidase to treat diabetes. The hypoxia-sensitive hyaluronic acid coupled with 2-nitroimidazole was used to self-assemble glucose-responsive nanovesicles [66].

4.4 Microneedle-assisted delivery of drugs for infectious disease

Controlled-release drug delivery systems might be used to provide antibiotics, which would lower posology (doses and frequency), helping the battle against antibiotic resistance [67]. MN has the potential to be a significant improvement in the treatment of infectious illnesses over parenteral administration. Tsioris et al. developed antibiotic-loaded silk microneedle. The release kinetics are assessed in an in vitro collagen hydrogel model, allowing

the model medicine to be tracked. Antibiotic-loaded silk microneedles are developed and utilized to show a 10-fold decrease in bacterial density afterward application. The study processing methodology may be applied to different silk-based structural formats for delivering drugs and biologicals [68]. Xueqing et al. developed dissolving microneedles for the delivery of antibiotics. The biomass of biofilms generated by *S. aureus* and *Pseudomonas aeruginosa* was decreased to 12.6% and 31.3%, respectively, in in vitro tests. In vivo, a study reveals that the -amylase-PDA@Levo microneedle (MN) could remove biofilms from infected wounds and kill exposed bacteria, lowering inflammation and aiding wound healing. However, mild photothermal therapy did not cause tissue damage [69]. Junhua et al. developed microneedle based for the treatment of bacterial biofilm. The findings indicate that microneedle-mediated treatment is more effective than drug-in-free for treating *Vibrio vulnificus* biofilms [70].

To establish a prolonged antibacterial impact at infection sites in wounds, Mir et al. designed carvacrol (CAR) poly (caprolactone) nanoparticles (NP) as a delivery method and incorporated them in dissolving MN. When comparing MN loaded with free-CAR (CAR-MN) and MN with CAR nanoparticles (CAR/NP-MN), the findings demonstrated that the concentration of CAR in the dermal layers was much greater when employing MN with CAR nanoparticles [71]. In another study, Lee et al. created bleomycin-coated MN by using a solid MN and a CMC solution containing the medication as a coating. In an ex vivo experiment using pig skin, the MN's bleomycin delivery efficiency was determined. After 10 min, the microneedles had delivered 74% of their bleomycin load. The skin absorbed 82% of the bleomycin when the insertion duration was prolonged to 15 min. The highest quantity absorbed into the skin was 82% after 15 min of implantation and did not rise further. Using an in vivo rat model, the authors compared the MN to standard SC injections to get a pharmacokinetic profile. The MN-derived bleomycin had a half-life of 36 min, whereas the conventional subcutaneously injected form had a half-life of just 8 min [72].

4.5 Microneedle for vaccine delivery

The epidermis includes many dendritic and Langerhans cells, both specialized immune cells, rendering transdermal delivery a potential immunization method. As the first line of defense in the immune system, Langerhans cells are responsible for initiating, processing, and presenting antigens to other immune cells [73]. Based on this anatomical fact, several forms of MN, such

as dissolving, hollow, and coated MN, have been used to administer vaccines. Ovalbumin (OVA) is a protein antigen often used in immunization investigations that use MN [74]. Nanoparticles carrying OVA and poly(I:C) were effectively delivered using a hollow MN created by Du et al. They showed in their study and compared the immunoglobulin G2a (IgG2a) antibody response elicited by the OVA/poly(I:C) solution in a mouse model with that caused by poly (lactic-*co*-glycolic acid) (PLGA) nanoparticles, liposomes, mesoporous silica nanoparticles, and gelatin nanoparticles, controlled drug delivery vehicles, including PLGA nanoparticles and liposomes, improved immune responses [75]. Kim et al. developed MN-containing vaccines against MERS-CoV-S1 and SARS-CoV-2 to induce antigen-specific solid IgG responses. MERS-CoV-S1 and SARS-CoV-2 trimeric recombinant subunit vaccines were generated with and without immune stimulants and included in a soluble CMC MN. These vaccines were examined in an in vivo mouse model for their immunogenicity after administration through MN or with a conventional SC needle. Vaccines against MERS-CoV-S1 administered by MN elicited more robust humoral responses than those administered via needle.

Furthermore, when vaccinations were administered by SC injection, incorporating the immunological sequences resulted in considerably greater IgG responses than without them. There were statistically significant increases in IgG responses after MN administration of either MERS-CoV-S1 or SARS-CoV-2 compared to preimmunization groups. Antibody titer was not noticeably affected by the use of immune stimulants in this instance. In addition, gamma radiation sterilization did not compromise the immunogenicity of MN vaccines, allowing for their safe use in clinical settings [76]. Peter et al. developed a microneedle coated with nano-capsule for vaccine delivery. Microneedle-mediated transcutaneous vaccination with interlayer-cross-linked multilamellar lipid vesicles carrying multilayers resulted in robust antigen-specific humoral immune responses with the balanced generation of multiple IgG isotypes. In contrast, intradermal injection of soluble or vesicle-loaded antigens or transcutaneous vaccination with microneedles encapsulating soluble protein resulted in weak, IgG1-biased humoral immune responses [77]. To successfully administer OVA in a mouse model, McCrudden et al. developed a dissolving MN that was constructed using poly (methyl vinyl ether-*co*-maleic acid) (PMVEMA). According to the findings, the MN could transport the active substance, which resulted in the activation of humoral and cellular responses. It showed an increase in the production of immunoglobulins and cytokines [78] (Table 1).

Table 1 Microneedle-based drug delivery of active compounds.

S. No.	Type of microneedle	Active drug	Method of fabrication	Special comments	References
1.	Dissolving MNs	Gentamicin	Molding	The prepared MNs penetrate into the skin layer and release the drug in a sustained manner.	[79]
2.	Dissolving MNs	Lysozyme	Droplet-born air-blowing method	The stability of lysozyme (LYS) as a model protein was maintained in polymer MNs by maintaining a low fabrication temperature and a gentle drying environment.	[80]
3.	Dissolving MNs	Parathyroid hormone	Micromolding	In a rat model of osteoporosis, the polymer MNs of PTH demonstrate outstanding efficacy for transdermal drug delivery, with relative bioavailability reaching $100 \pm 4\%$ compared to normal injection.	[81]
4.	Dissolving MNs	Vitamin D3	Laser engineered micromolds	The polymer MNs exhibit high levels of mechanical strength and may be placed into the skin risk-free. In addition, the manufactured vitamin D3 as a nanoparticle offers a quick release profile of the drugs.	[82]
5.	Dissolving MNs	Fluorescein sodium and fluorescein isothiocyanate–dextran	Laser-engineered silicone micromolds	The prepared MNs enhance the drug release profile.	[83]
6.	Dissolving MNs	Sulfonhodamine B	Casting	The prepared MNs showed a significant impact on drug release. The MNs improve the drug release profile	[84]

Continued

Table 1 Microneedle-based drug delivery of active compounds—cont'd

S. No.	Type of microneedle	Active drug	Method of fabrication	Special comments	References
7.	Dissolving MNs	Insulin	Molding	Polymer MNs that were made using alginate and hyaluronate had excellent mechanical properties and also had a favorable degradation rate for the release of insulin.	[85]
8.	Coated MNs	Bleomycin	Molding	Compared to intralesional injection, bleomycin-coated MNs can provide a more concentrated medication dosage.	[72]
9.	Bioresponsive and biodegradable MNs.	Doxorubicin	Microtransfer molding and cross-linked by UV irradiation	The behavior of MNs in terms of drug release may be affected by the degree of cross-linking present in the polymer.	[86]
10.	Hydrogel-forming MNs	Diclofenac sodium	Molding	MNs prepared with chitosan and PVA had increased mechanical strength and prolonged drug release.	[87]
11.	Solid MNs	Insulin	Molding	Polymer solid MN pretreatment creates skin microchannels that slowly distribute insulin to reduce blood glucose.	[88]
12.	Dissolving microneedles	Capsaicin	Drawing lithography	This method may transport lipophilic drugs of varied lipophilicity and nano-sized, high-molecular-weight, low-bioavailability drugs.	[89]
13.	Dissolving microneedles	Artemether	Micromilling technique	MNs provided a potentially less invasive route for the systemic distribution of drugs with poor water solubility.	[88]

14.	Dissolving microneedles	Interferon-alpha	Micromolding method	The results indicate that MNs are a potential biomaterial device for the treatment of protein medications, and they may improve patient's quality of life by sparing them the discomfort of injections.	[90]
15.	Dissolving microneedle	Amphotericin-B	Silicone molds	According to the findings, the device demonstrated excellent repeatability, tensile robustness, faster dissolving, and full drug retrieval.	[91]
16.	Dissolving microneedle	Besifloxacin	Micromolding technique	It was noticed that MNs get disintegrated when they were in the cornea. When compared to the use of free besifloxacin liquid, the use of MNs for 5 min significantly increased the amount of besifloxacin that was deposited on the cornea, and that was able to penetrate it.	[92]
17.	Dissolving microneedle	Cisplatin	Two-step centrifugation casting method	To treat cancer in a locoregional area, the MN may be implanted entirely under the patient's skin, and the compound-carrying points could be inserted directly into the cancerous tumor.	[93]
18.	Metal-organic framework microneedle	Nitric oxide	Template replication method.	The authors demonstrate that it can accelerate vascularization, tissue regeneration, and collagen deposition, indicating a promising future application in wound healing and other therapeutic settings.	[94]

5 Conclusion

Wounds are a rising problem in healthcare systems all over the globe. They may be excruciatingly painful, impose financial difficulties, and they can drastically diminish patients' quality of life. They encompass a variety of ailments, such as diabetic foot ulcers, venous ulcers, pressure ulcers, and burn injuries. To combat such a severe health issue, immediate therapy is required immediately. Parenteral, inhalational, oral, and transdermal administration are only some ways to deliver therapeutic active compounds to the wound bed. It is possible to circumvent many of the issues that correspond with other traditional procedures by administering the therapeutic active compound directly via the skin. A few examples of these issues include gastrointestinal discomfort, first-pass metabolism, pain, and difficulties with dosage effectiveness. The transdermal approach using MNs stands out as a potentially useful option among these strategies.

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CHAPTER 10

Microneedle for delivery of cosmeceuticals and enhancing skin appearance

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1 Introduction

The environment, mechanical injury, infection, and aging all cause the skin to deteriorate during a person's lifetime. Solar radiation of different wavelength regions like IR, UV, and visible along with polluted air, cigarette fume, less nutritious food, and certain less well-studied elements are the main groups of environmental factors that affect skin aging and malignancy [1]. When our skin gets exposed to ultraviolet rays, it can age prematurely and develop sun damage symptoms like actinic keratosis, solar elastosis, leathery skin, and liver spots. According to numerous studies, some practices that expose people to the sun, such as sunbathing, can lead to basal, squamous cell, and melanoma skin cancers [2].

The DNA in our skin cells can be harmed by excessive UV radiation from the sun or sunlamps. DNA explains to our cells how to operate. Skin cancer may develop if enough DNA damage accumulates over time and triggers cells to begin proliferating out of control. The damage to the skin from air pollution is caused by an increase in oxidative stress. The disruption in the equilibrium between the generation of highly reactive oxygen species or free radicals and defensive antioxidants is referred to as oxidative stress. Those reactive oxygen species are neutralized by antioxidants, which stop oxidative damage to cells and tissues. Both enzymes and nonenzymes make up the cutaneous antioxidant system. Glutathione peroxidase, catalase, and superoxide dismutase are three examples of enzyme antioxidants.

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Antioxidants that are nonenzymatic or have low molecular weight also help to keep cellular redox balance. Multiple hormones, including estradiol and melatonin, as well as various vitamins, including E and C, are grouped together in this section. Reactive oxygen species and some free radicals are produced, which react with the plasma membrane that is rich with lipids, to start the lipid peroxidation reaction cascade. A vicious cycle is created when some oxygen species, which are highly reactive, encourage the synthesis of pro-inflammatory mediators, which in turn causes an aggregation of some phagocytic cells and neutrophils, which in turn produces more free radicals. Oxidative stress triggers intricate biological reactions that cause gene-related damage, the activation process of signaling pathways, and transcription factors including extracellular signal-regulated kinases, which are involved in the development and differentiation of cells as well as the breakdown of dermal connective tissue.

Through oxidative damage, air pollutants cause severe modifications to the proteins, lipids, and deoxyribonucleic acid that make up human skin. It causes outside cuticle aging and inflaming conditions like psoriasis, atopic dermatitis, acne, contact dermatitis, and skin cancer [3]. Many antioxidants, including tocophenols, flavonoids, and carotenoids as well as vitamins (A, C, D, and E), certain lactobacilli, essential omega-3 fatty acids, and proteins are those which have been identified as agents capable of improving human health and appearance of the skin. Our diet's deficiency in essential nutrients causes signs of aging in our skin [4]. Loose facial skin is a troublesome aging symptom. Surgery used to be the only option for correcting looseness. Although surgical lifting is still the gold standard, people are increasingly asking for less invasive procedures.

Surgery complications include hematoma, which is basically a collection of blood under the skin, scarring, nerve injury, and hair loss. In rare cases, a face-lift can also abrupt the blood supply to facial tissues. This can have worse outcomes like skin loss. More and more patients are looking for operations with minimum to absolutely no recovery time, lower risk profiles, and a more natural appearance [5]. The main barrier to medication delivery via the intramuscular method is skin. The exterior stratum corneum, the thickest of all, and the intermediate epidermis, the dermis, make up the three primary layers of skin. Only certain molecules, such as drugs with low molecular weight and lipid-based compounds, can get through the stratum corneum layer, which acts as a major obstacle. The main issue with transdermal technology is that many medications cannot penetrate the skin quickly enough to exert their therapeutic effects. Microneedles, a

sophisticated technique created by researchers, enable the puncturing of hydrophilic, higher molecular weight substances into the stratum corneum. When medications are administered using a microneedle device, excess drug molecules can penetrate the skin because the medication can pass across the stratum corneum layer. The speedier start of action, higher acceptance by patients, self-administration, improved permeability, and effectiveness of this technique are its unique features. In addition to better medicinal advantages, microneedles produce outcomes that are extremely precise, and repeatable [6].

2 Structure of the skin

The skin is the body's major organ, accounting for greater than 10% of total body weight. It also allows the body to communicate most directly with its surroundings. The stratum corneum, remaining epidermal layers, dermis, and subcutaneous tissues make up the four basic layers of skin.

The fundamental job of the epidermis is to create the defensive, semi-permeable stratum corneum that makes it possible for life to exist on land by limiting water loss, ensuring that the skin is adequately hydrated, and guarding against overhydration. The patterned lipid lamellae located in the extracellular gaps between corneocytes serve as the curbing role of the stratum corneum. Much of the stratum corneum, the epidermis' topmost layer, is made up of corneocytes, terminally developed keratinocytes. Keratinocytes are those cells, which contribute to the major part of the epidermal layer. They comprise a variety of functions that are vital for skin restoration. They perform the re-epithelialization procedure, in which keratinocytes move, multiply, and differentiate to re-establish the epidermal barrier. Re-epithelialization is the process, by which a wound is covered in fresh epithelium. Successful wound closure depends on the cellular and molecular mechanisms involved in epithelialization's onset, maintenance, and completion [7]. The epidermis, which is a vital part of the human body, not only gives the immune, nutritive, and other sustenance systems for the epidermis, but it also regulates pain, pressure, and temperature. A coarse reticular layer is a term used to describe the dermis's primary structural element.

The dermis is between 0.1 and 0.5 cm thick and is made up of elastic connective tissue, which provides elasticity, and collagenous fibers, which provide 70% of the framework and padding for the dermis. The dermis has a minimal cell population in general. A large vascular network that is located

within the dermis supports the skin's needs for nutrition, healing, and immune responses as well as the remaining of the body's needs for immune response and thermal control. The primary cells are fibroblasts, those which create the regulatory connective tissue constituents.

The subcutaneous tissue lies at the bottom among the skin layers, which can also be called the hypodermis. The hypodermis serves as an area for energy storage, tension absorption, and heat insulation. This layer is made up of a web of lobulated fat cells attached to the skin layer dermis by fibrous proteins like elastin and collagen [8].

The stratum corneum is the topmost layer of the skin. It is to blame for the restricted permeability of molecules. Since it can prevent the passage of foreign things, including drug molecules, this deceased coating of tissue acts as an especially efficient barrier [9].

It was considered to (i) keep away from problems with emptiness in the abdomen, pH effects, also enzyme suppression related to the gastrointestinal region, (ii) avoid liver-related initial metabolism, and (iii) sanction regulation of input, as demonstrated by stopping drug administration through eliminating that device, the cutaneous layer developed as a capable area for administration of drugs.

The transdermal drug administration system has a few drawbacks as the best candidates for this procedure are only strong medication. Some patients may experience skin discomfort where the application was made. The delivery method is ineffective for medications that require high blood levels. The system is expensive. Drug binding to the skin may cause dose dumping. It can only be used for chronic disorders; it cannot be used for acute conditions since chronic conditions call for long-term pharmacological therapy, such as diabetes, angina, and hypertension. Cutaneous Metabolism Affects the System's Therapeutic Performance. Ionic medications are not good candidates for transdermal procedures [10].

Among all the possible methods for transdermal drug delivery, nanocarrier- or MN-based drug delivery systems are painless, non-invasive, or minimally invasive and need no expensive equipment, as opposed to conventional transdermal delivery techniques. More significantly, they can add more sophisticated features including improved theragnostic capabilities, regulated medication release rates, and skin penetration efficiency.

Skin's structure goes through a lot. But a vast size of the population is still more concerned about the aging process. To find an efficient antiaging treatment, it is needed to understand what aging does to our skin. Protection is the epidermis' primary role, which is accomplished by the development of a

stratum corneum. The keratinocytes still exhibit particular differentiation products linked to the keratinization process even though the epidermis atrophies with age. Both the young and exposed old skin have a stratum corneum that was well-formed. Age does not appear to affect the ability of the epidermis to produce a typical stratum corneum from a morphological standpoint. Physiological research on skin permeability indicates that the ability to limit water loss is not affected in old skin, which supports this hypothesis. The junction between the dermis and epidermis gets altered, but the aging epidermis shows only minor changes. In the young, the surface area of the junction between the dermis and epidermis is increased by the widespread interdigitation of basal cells with the dermis, which also securely attaches the epidermis to the dermis to resist shearing forces. A flattened dermal-epidermal junction, on the other hand, would produce a more delicate tissue that was less resilient to tearing forces. Because of the flat dermal-epidermal contact in aged skin, the epidermis is frequently susceptible to being peeled off by trauma. In scar tissue, comparable flat dermal-epidermal connections have been observed. This discovery also explains why suction blisters can be generated on aged subjects considerably more quickly [11]. In the microneedling method, the multiple microprisms produced during tiny punctures with thin needles are employed to generate controlled inflammation in the skin. Microbleeding can occur when the skin is punctured with needles of the proper length, which can harm the skin's surface blood vessels. This initiates a procedure similar to the healing process for wounds. Just below the epidermis, microneedling produces minute fractures in the blood vessels. Blood platelets, which are necessary for blood clotting, are released during this phase, and collagen, elastin, and growth factors synthesis are encouraged [12].

The mechanism of microneedling has been possible only because of the strong immunity system of the skin. Due to its location and size, it can receive a variety of signs from the environment and examine those on the basis of danger to select the best course of action: one of two create tolerance or a quick immunological reaction. The dermis's predominant cell type is fibroblast. Fibroblasts take a role in cutaneous immunological reactions. In addition to acting as amateur antigen-presenting cells, keratinocytes and fibroblasts can communicate with immune cells and one another in the skin via secreting cytokines and chemokines. Because these are proteins, the proteinases in the environment regulate their availability, activity, and selectivity when these substances are expressed on the membranes of the cells, discharged by the cells, or transported by exosomes [13,14].

3 Microneedling technologies: An evolutionary step toward microneedle usage

One of the most prevalent and emotionally taxing issues in the practice of dermatology is acne scarring. They stand above the surface of the surrounding skin and are caused by too much collagen during healing. One of the most prevalent skin conditions is acne vulgaris. 80% to 85% of teenagers and young adults are affected by it. Up to 7% of mature individuals may continue having acne well into their mid-30s or 40s. A condition known as acne affects the pilosebaceous unit and is characterized by irregularities in sebum synthesis, follicular epithelial desquamation, bacterial growth, and inflammation. The scar treatment aims to improve the physical look of the skin. Techniques for resurfacing, removing the epidermis, and enabling reepithelialization by collagen remodeling. These consist of dermabrasion, lasers, cryotherapy, selective photo thermolysis, chemical peels, and electrosurgery. Surgery techniques include subscision, punch elevation, and excision [15].

Ablative or nonablative techniques of treatment are available for various kinds of acne scars. Nonablative therapy involving lasers and light, such as intense pulsed light (IPL), are now a popular treatment choice since ablative techniques like dermabrasion and laser resurfacing create major postoperative alterations in the skin and need lengthy recovery times. An ablative therapy (microneedle therapy system) is a straightforward technique that can rapidly and accurately puncture any skin when used with the appropriate equipment. The method can be applied to locations that are not good candidates for laser resurfacing or peeling [16].

The idea of utilizing arrays of miniature needles for medication administration dates back to 1976, but it was not until the late 1990s when high-precision industrial microelectronic techniques were used that the production of such devices became feasible. As a result, microneedle manufacturing technology has advanced, attracting considerable interest from the academic community as well as the pharmaceutical business. It now helps to reduce cellulite, stretch marks, fine lines, wrinkles, and acne scars while also enhancing skin firmness, moisture, and texture. The utilization of microneedling fractional radiofrequency (MFR) in acne therapy is a novel strategy that has been demonstrated to be therapeutically effective in controlling acne scars without endangering the epidermis.

The three steps of the wound-healing response initiation/inflammatory, proliferation, and remodeling are the basis for microneedling, which is

consequently based on controlled mechanical stimulation of these phases. Briefly, phase I of the rehabilitation process involves the recruitment of neutrophils and platelets toward the damaged site, which activates the release of growth factors, which all assist in creating and propagating intercellular matrix proteins. In the second phase, growth factors keep exerting their influence on fibroblasts, keratinocytes, and monocytes. A fibronectin matrix is created as a result, and fibroblasts eventually deposit collagen. Skin tightening occurs in phase III as a result of collagen's conversion to collagen I via tissue restructuring and vascular maturation [17].

The four varieties of MNs that may be broadly categorized based on morphology are coated microneedles, hollow microneedles, and solid microneedles dissolving microneedles. As the name suggests, solidified microneedles are essentially microneedles that have been solidified with the goal of enhancing the diffusion of biotherapeutics after pretreating them. Solid microneedles typically develop micron-sized holes that enable medications to gently diffuse into the skin, increasing effectiveness. Medications are typically coated on the Coated Microneedles utilizing biocompatible polymers. The therapeutic is released into the skin layers when the device is put into the skin and the coating dissolves or degrades. Dissolving MNs are biodegradable polymer-based MNs that include medicines in a capsule. The encapsulation instantly dissolves and releases the medicines after being put into the skin, leaving no residue behind in the skin. Different types of microneedles and their mechanism of action are described in Fig. 1.

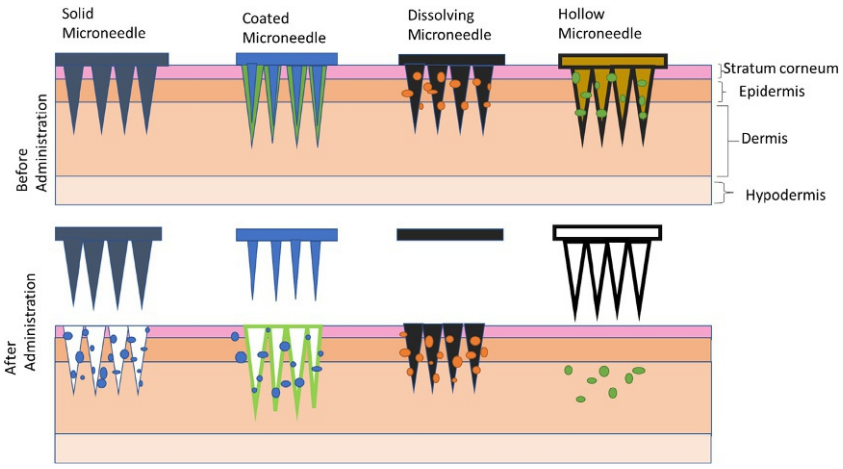

Fig. 1 Different types of microneedles and their mechanism of action.

Microneedles have uses for many purposes including small molecule delivery, anti-cancer therapy, anti-diabetes therapy, and cosmetics. Most of microneedle's applications include the delivery of drugs, particularly vaccines. But recently, there has been a lot of attention paid to diabetes treatment as well as therapy for cancer.

4 Application of various kinds of microneedles

4.1 Solid microneedle

The majority of solid microneedles' actions have focused on pretreating the epidermis to increase the permeability of medications with lower molecular weight. To get past the Stratum Corneum layer's structural barricade, it used to be helpful to prepare a passage on the skin layer using a solid microneedle. In comparison with medications with high molecular weight, posttreated tiny molecules can readily enter the skin layer via the skin pore. Contrary to a traditional solid microneedle patch, roller-type microneedles have been created, such as the FDA-approved dermaroller and other commercially available gadgets. Those materials have also been employed for optical skin cleansing in addition to creating a channel for transdermal medicine distribution on the skin layer. For the distribution of cosmetic products and medications, another microneedle that has been commercialized has been created. Prior to applying for acne medicine, this nanomedical gadget was utilized to treat the skin, drug exhibits improved pharmacokinetic effects.

Solid microneedles have also been used in gene expression studies; the transported gene was successfully expressed in ex vivo tests on human skin. Large molecules have been delivered more effectively using solid microneedles along with sonophoresis. The transport of medication through the skin while being impacted by ultrasound is known as sonophoresis. Usually, a pressure wave with a low frequency of under 100 kHz is used in this method. The stratum corneum lipid bilayer can be broken by ultrasonic applied to the skin. As a result, it is simpler for medication molecules to pass through the skin [18]. High permeability, greater drug molecule efficacy, and little tissue injury compared to conventional injection procedures are the main effects of employing solid microneedles on the skin.

4.2 Coated and hollow microneedles

Both coated and uncoated microneedles were utilized to provide lidocaine as local anesthesia. According to this interpretation, the skin's level of

lidocaine was high enough to produce long-lasting local anesthesia. Although both traditional and microneedling methods of delivering lidocaine produced the same level of local anesthesia, the individuals felt much less pain with microneedle delivery and strongly preferred it to intradermal injection with a hypodermic needle [19].

Numerous experimental findings also show the possibility of licensed influenza subunit vaccines delivered by microneedle, which may help to improve vaccine reach and protection while decreasing mortality caused by influenza globally.

The latest invention in gene silencing by siRNA therapeutics targets malignant, hyperproliferative, or disabling skin disorders brought on by irregular gene expression. To be therapeutically successful, therapeutic siRNAs have to be stable and functional enough to penetrate via viable skin layers, which is commonly acknowledged by doctors and patients as a non-invasive manner that increases patient compliance.

The fundamental benefit of employing coated microneedles is that they retain their strength even after the coating has been dissolved, and the dosage is also extremely accurate. The coating needs to be correct for enhanced coated microneedle-based medication delivery. The formulation of the coating and its components are crucial in this regard. There is no skin injury following application because the coated microneedles are constructed of biocompatible polymers. The measles vaccine has also been administered using coated microneedles. The ability of the measles vaccine to be stabilized on microneedles and its effective reconstitution in vivo to produce neutralizing antibodies comparable to those produced by subcutaneous injection.

The coated drug or chemical of the coated microneedles must be entirely liberated from the microneedles and dissolved with the help of the interstitial fluid of the skin for successful drug delivery. Therefore, a key component of the optimum drug delivery of microneedles is the quick breakdown of the medication from the needles. Additionally, the microneedle's property of quick disintegration may increase patient convenience. Prior versions of coated microneedles required a lengthy skin application period (of about 10 min) to stimulate activity. The discomfort and unwanted skin stimulation that might result from long-term microneedle administration to the skin can also affect how well the medicine is absorbed.

In an effort to speed up skin application, different coated microneedle formulations have recently been researched. In the application for the creation of microneedles with safe and quick disintegration. Here, a formulation for quick disintegration was created to shorten the time microneedle

needs to be applied. The glycerol, polymers, Tween 80, Brij, and croscarmellose sodium were used as thickeners, plasticizers, disintegrating agents, and surfactants in the development of the rapid disintegration-coated microneedle. The kind and quantity of polymer have a significant impact on the effectiveness of coating and medication release.

Instead of polyvinyl alcohol (PVA), HPMC might be a good material for quickly destroying microneedles. Additionally, the HPMC and PVA microneedles did not cause cutaneous toxicity. Poly(2-(diisopropylamino) ethyl methacrylate-b-methacrylic acid), a pH-induced charge-invertible polymer, was used in a study to layer-by-layer coat microneedles and speed up application time (to within 1 min). The coating efficiency, coating effectiveness, physicochemical properties, and disintegration properties of the coating solution would all be considered to maximize the drug administration efficacy of coated microneedles. Therefore, it is important to first look into the relationship between drug delivery effectiveness and the different coating solution compositions. Additionally, while using coated microneedles in clinical settings, the coating solution's safety should be taken into account.

Therefore, it was attempted to use the oral disintegrating film (ODF) enabling quick delivery of the drug, easy coating solution production, secure microneedle profile, and optimized coating solution composition. ODFs are known as the quickly disintegrated formulations in the pharmaceutical industry. ODF must dissolve in water within 3 min according to the European Pharmacopoeia, while FDA recommendations suggest that the in vitro disintegration time be less than 30 s. So, the key component of ODFs is their quick disintegration time.

ODFs are made with a variety of substances that encourage quick disintegration in the oral cavity, including thickeners, plasticizers, surfactants, and disintegrants. Thickener, one of the components, plays a significant effect on the rate of disintegration. Thickeners regulate the elasticity, tensile strength, and viscosity of ODFs and are connected to the film's breakdown, absorption, and dissolution. The development of the ODF has made extensive use of the polymer as a thickener. The usage of biodegradable polymers is associated with minimal toxic nature regarding safety.

Biodegradable polymers are ideal for application in tissue engineering or medication delivery systems because they break down quickly in the human body. Both polylactic acid (PLA) and polylactic-*co*-glycolic acid (PLGA) are commonly employed as low-toxic medication carriers. Therefore, to enhance the physical characteristics of ODFs, biodegradable gel-forming

polymers have been employed as thickening agents. PVA is a hydrophilic polymer that is one of the least toxic and most biodegradable of all polymers. It also has the potential to swell by absorbing moisture. Additionally, hydroxypropyl methylcellulose (HPMC) is nontoxic, water-soluble, and easy to use. Both polymers have the characteristic of becoming more viscous as the concentration rises.

Thus, it was suggested that the microneedle coating solution might be coated with the ODF formulation for quick disintegration. For the microneedle coating process, the solution used for coating must be taken into account. As a result, it was required to screen thorough evaluation by creating different concentrations of ODFs that could be coated and quickly dissolve. The DCS (disintegrating coating solution) for the microneedle coating was produced in this study utilizing either PVA or HPMC. Rapid DCS formulation was created for the quick disintegration of drugs from the microneedle, and DCS was subsequently mounted on the microneedle. Additionally, the dermal safety of DCS microneedle has been assessed to see whether skin application may lead to the creation of a safe and quick disintegrating microneedle [20].

4.3 Dissolving microneedle

Due to their great biocompatibility and safety, sugar-based dissolving microneedles are recognized as cutaneous biotherapeutic delivery. It would be incredible to employ sugars as dissolving microneedles since they can help maintain stability, making them an excellent delivery vehicle for different kinds of biotherapeutics such as nucleic acids, proteins, and peptides. One analysis assessed the usefulness, acceptability, and skin tolerance of human dissolving microneedle patch delivery. The skin accepted the microneedle patches well, developing a slight erythema that subsided completely in 7 days without causing any discomfort or edema. Without the use of an applicator, microneedle patches were applied by hand, and both investigator- and self-administration had comparable distribution efficiencies. There was a major part of the population, individuals were partially or completely sure that individuals applied the patch appropriately by themselves, and the patch administration was not uncomfortable at all. The use of microneedle patches rather than traditional injection with a needle and syringe was overwhelmingly preferred. Overall, the findings in this research show the viability of employing disintegrating microneedle patches for self- and investigator-administration for future applications in the deliverance of vaccines and

drugs in a preferred and well-tolerated approach that presents a compelling alternative to conventional hypodermic needles [21].

It is known that horse oil is added to the oil phase formulation because it helps enhance moisturization and skin barrier restoration. To reduce lateral wrinkles, adenosine, that often used to make cosmetics, was initially contained in dissolving microneedles. Skin repair and wrinkle reduction have been accomplished using dissolving microneedles. Even while lipophilic substances, like ceramides or natural oils, improve the skin barrier, it is difficult to distribute them using dissolving microneedles due to manufacturing issues.

In the initial studies, a topical medicine was employed and a dissolving microneedle patch to administer the medication in two stages, which included microneedles loaded with a hydrophilic compound and lipophilic formulation, to enhance skin's barrier integrity and the effectiveness of drug deliverance. In the same patch, horse oil spread and adenosine-packed dissolving microneedle arrays were created. To verify that the compositions were successfully delivered, in vitro testing was done. To evaluate the procedure's efficacy, horse oil was efficiently delivered via the skin across the microchannels created by the Adenosine-dissolving microneedles.

After that, it was discovered that when compared to Adenosine-dissolving microneedle patches, horse oil-adenosine-dissolving microneedle patches considerably reduced wrinkles, elasticity, and dermal density. No unfavorable incidents were noticed. They tested horse oil-adenosine-dissolving microneedle patches in vitro and in humans for skin repair and wrinkle reduction. The outcomes supported simultaneous hydrophilic components (horse oil and adenosine) and lipophilic transdermal transport and effectiveness. This secure and effective combination patch technology may find widespread use in transdermal therapy for both medicinal and cosmetic purposes [22].

According to a recent study, standard dissolving microneedle patches and cream formulations can be used in combination to transport more encapsulated medications into the skin layer, increasing their effectiveness in people. The cream is applied first in traditional combination procedures, which have significant drawbacks in terms of delivery effectiveness and time. It is challenging to reach the appropriate interval for users since the components in topical formulations can vary, which could impact the drying rate.

Additionally, applying dissolving microneedle patches for a longer or shorter duration of time did not have a synergistic impact on drug delivery.

These restrictions were anticipated to be solved by rearranging the topical and dissolving microneedle applications, as stated in this combinatorial application. Adenosine-dissolving microneedle patches were used to first establish skin channels for the transport of the encapsulated medicines and later, the ingredients of cream formulations. The maintenance of skin perforations up till the application of the adenosine cream in the combinatorial application was a crucial component in the successful delivery of the adenosine in the cream through cutaneous pathways.

After removing the patch, applying the cream formulation would enable the delivery of adenosine through cutaneous pathways. Furthermore, it was anticipated that fewer adenosine may be given over time since the skin will ultimately regenerate. In the traditional method, as it is known, there is a requirement of interval for the drying of liquid formulations while with this combinatorial application, no interval of time was required. This referred to an improvement in convenience and safety over solid microneedles.

In contrast to the more typical cream-only administration, the combination therapy using Adenosine-dissolving microneedles and topical cream offered better drug distribution and efficiency. Microchannels were made by dissolving microneedle arrays, which were used to distribute both encapsulated medications and cream ingredients. This combinatorial application was demonstrated in an objective clinical experiment to be more effective for the reduction of dermal density, wrinkles, hydration, and elasticity, using an antiwrinkle chemical, adenosine. As user-friendly and non-hazardous materials make up dissolving microneedles, this unique approach offers a secure and ground-breaking breakthrough for the improvement of transdermal therapy [23].

Additionally, for skin discoloration and hydration treatment, dissolving microneedles provide cosmetic active substances like retinyl retinoate, ascorbic acid, 4-n-butylresorcinol, and epidermal growth factor. Hyaluronic acid has long been employed as a material for a matrix with a variety of purposes since it can be readily manufactured into microneedles and serves as a normally existing macromolecular element in the human body. The effectiveness of these dissolving microneedles that contain cosmetic active ingredients is often evaluated in contrast to a control dissolving microneedle that contains no active ingredient.

That comparison with an ordinary blank dissolving microneedle displays the impacts of the active chemicals carried by dissolving microneedles and hides the improving impact produced by the skin's hyaluronic

acid that retains and absorbs water to keep the skin moisturized and supple. The molecular mass of hyaluronic acid influences its water-holding characteristics; the greater the molecular mass, the more intense the water-holding properties. Furthermore, the more time that hyaluronic acid stays in the human body, the better its ability to resist hyaluronidase, the enzyme that degrades hyaluronic acid. Higher molecular mass Hyaluronic Acid has already been employed as an initial ingredient for filler materials as well as an intraarticular injectable treatment in the medical and cosmetic fields.

The demand for manufacturing dissolving microneedles with higher molecular mass Hyaluronic acid is enhanced because further skin improvement impact is anticipated in addition to active component benefit. However, the high viscosity of hyaluronic acid, which makes it difficult to handle and hence form the microneedle, makes the manufacture of dissolving microneedles a challenge. To avail the benefit of the hyaluronic acid's large molecular weight, connected hyaluronic acid was employed to make dissolving microneedles; however, only a continuous releasing profile of its active ingredients was examined.

To enhance the therapeutic effects of dissolving microneedles over the role of the dissolving microneedles matrix, the influence of Hyaluronic Acid on skin health must be evaluated. Dissolving microneedles contain a considerable amount of hyaluronic acid, which is important for maintaining the skin's hydration and elasticity. In these studies, the clinical skin improvement effects of adenosine-covered low molecular mass hyaluronic acid dissolving microneedle patch (Ad-LMN) and high molecular mass hyaluronic acid dissolving microneedle patch (Ad-HMN) were compared.

Following an in vitro skin infiltration study with both Ad-HMN and AdLMN patches, the enhancement in dermal thickness, elasticity impact, and skin wrinkling, in human crow's feet was assessed in randomized medical treatment. It was also determined whether administering adenosine to the Ad-HMN and Ad-LMN groups was safe. It was also examined if it was safe to provide adenosine to the Ad-LMN and Ad-HMN groups. For clinical studies, the Ad-HMN group outperformed the Ad-LMN group in all metrics, and both groups displayed wrinkle reduction lasting up to 4 weeks following treatment without any skin adverse effects. As a result, it is anticipated that the Ad-HMN patch will reduce skin wrinkling, density, elasticity, and wetness without causing any sensitive or uncomfortable side effects [24]. Various applications of different types of microneedles are highlighted in Fig. 2.

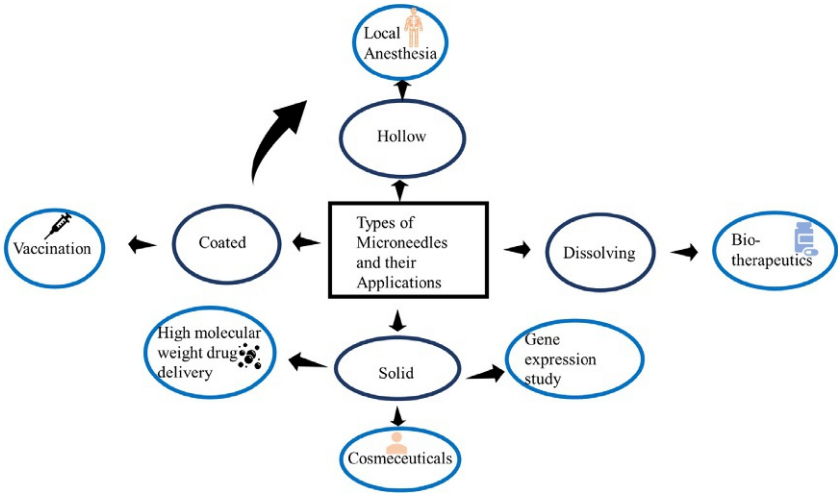

Fig. 2 Various applications of different types of microneedles.

5 Different applications of microneedling in dermatology

Dermarollers have been utilized for a variety of purposes, and numerous studies have provided proof of their value. With the use of microneedling, the dermaroller uses collagen induction to treat wrinkles and facial rejuvenation along with acne scars, and stretch marks. The conventional dermaroller version and the small variants are the two available variations. The dermaroller works better on boxcar-style and rolling scars [25,26].

Dermaroller is an easy-to-use product. As in the case of acne scars, it is a simple procedure. Topical anesthesia is applied to the region of treatment and left on for 45 min to 1 h. After the region has been rolling 15–20 times in all directions, pin-point bleeding that occurs can be readily stopped. Following treatment, saline pads are used to hydrate the region. Depending on the dimensions of the region which needs to be treated, the total operation takes 15 to 20 min. It is advised to wait at least 6 weeks between treatments because it takes a while to form new collagen naturally. For moderate acne scarring, three to four treatments might be required [27].

5.1 Skin regeneration

By minimizing fine lines and wrinkles, shrinking pores, increasing suppleness, and increasing elasticity, this percutaneous collagen induction gives the skin a younger-looking appearance overall. When the technique is done with topically applied vitamin C serum and tretinoin, the benefits are

improved. In order to significantly reduce wrinkles and pigmentation, microneedling has also been coupled with endothelial precursor cell conditioned media generated from human embryonic stem cells [28]. In a significant multicenter trial, fractional microneedling radiofrequency was investigated and shown to be efficient in decreasing wrinkles [29].

5.2 Scars

The postacne facial atrophic scars are the most typical use for microneedling and numerous trials have been carried out to assess it both on its own and in conjunction with additional therapies like platelet-rich, subscision, plasma chemical peels, cryotherapy, and technique. In comparison to ice-pick scars, rolling and boxcar scars have been reported to respond better to microneedling. With little downtime, it is risk-free for all skin types. There is little chance of postinflammatory dyschromia, and just the affected area needs to be treated. However, for a noticeable improvement, a minimum of 4–6 sessions are needed. Nearly all types of surgical scars have responded well to microneedling treatments. Even burn scars have been shown to be reduced by microneedling [30].

5.3 Acne vulgaris

The skin's pilosebaceous unit, which consists of hair follicles connected to oil glands, is afflicted by acne. Inflammatory lesions (papules and pustules), noninflammatory lesions (open and closed comedones), Seborrhoea (excess grease), and varied levels of scarring are some of the clinical characteristics of acne. The areas of the body with the largest density of pilosebaceous units are the ones where acne is most common like facial, neck, upper chest, shoulders, and back. Severe nodulocystic acne is characterized by nodules and cysts [31]. With the introduction of fractional microneedling radiofrequency, the use of microneedling for acne vulgaris has also increased. This aids in lowering sebum production by directly targeting the sebaceous glands. It is also known to lessen keratinocytes' hyperproliferation.

5.4 Androgenic alopecia and alopecia areata

Androgenetic alopecia commonly known as male-pattern hair loss or common baldness in men and female-pattern hair loss in women, is a medical condition that affects both genders. The pattern of hair loss is well-defined, androgen-dependent, and heritable. It is believed that androgen-stimulated hair follicle miniaturization targets those hair follicles predisposed genetically, which causes the gradual replacement of big, pigmented hairs with

scarcely perceptible, depigmented hairs in afflicted areas [32]. Autoimmune, inflammatory hair loss on the scalp or body is known as alopecia areata. There are two distinct subgroups of individuals with this condition: those with complete loss of both body and scalp hair during the ultimate stage of the disease [33]. One of its most recent advancements is the use of microneedling on the scalp for alopecia. When it is compared to only minoxidil by itself, it has been discovered that the combination is superior [34]. Patients who take minoxidil are given prescriptions for home-use dermarollers, and better hair growth is shown. In the case of alopecia areata, microneedling has also been paired with topical triamcinolone acetonide treatment, and improved results have been seen [35].

While there have been few studies on this hair loss therapy, it can be proven to enhance hair follicle development when paired alongside additional hair growth therapies such as platelet-rich plasma, topical steroids, and minoxidil [36].

5.5 Pigmentation

The Dermafrac technology has made microneedling to the periocular skin possible. To treat melasma and periorbital hypermelanosis, microneedling has been used with different skin-lightening products which include chemical peels. Melasma is a pigmentary condition of the face that often affects the upper lip, forehead, and cheeks. 90% of all cases of this illness are in women, who also have a higher prevalence [37]. Improved topical infusion and cutaneous microinjury are made possible by DermaFrac's special blend of technologies. A handpiece with varied tip caps is rolled over the skin, putting it under a vacuum and putting it in contact with hundreds of microneedles that pierce the outermost layer of skin and reach the top layer of the papillary dermis simultaneously administering an injectable solution to the region [38].

5.6 Laxity

Skin laxity is mostly treated with microneedling. It helps to tighten skin after liposuction and with loose skin on the arms, belly, neck, thighs, and places between the breasts. New-generation microneedles are a specific subclass of microneedles with nanopatterned surfaces for improved therapeutic efficacy and delivery. By encasing near-infrared (NIR) absorbers, silica-coated lanthanum hexaboride (LaB6@SiO2) nanostructures, and the cargo molecules that are delivered in compostable polycaprolactone (PCL) microneedles, scientists developed novel concepts of transdermal delivery systems activated by NIR light. Due to the encapsulated nanomaterials' properties as NIR

absorbers, heat-induced degradation of the PCL Microneedles will help the active medication to be released through the skin. By controlling NIR exposure, the release pattern may be altered. Most solid microneedles' rate-limiting stages are related to microneedle size and hydrophobicity. Nanomaterial-coated microneedles have lately been used to overcome these difficulties [39].

6 Approved microneedle devices

6.1 Dermaroller

PCI is accomplished by applying numerous needles. To do this, roller devices with needles that project have been created. The FDA-approved dermaroller is a hand-held device containing microneedles manufactured of medical-grade solid steel that emerges out of a cylinder roller. Directly on top of the skin, the rolling mechanism is used in all directions. When the microneedle arrangement had been set to a specific fast rate, skin piercing employing microneedles of comparable lengths was successful. In this case, the velocity at which the microneedle insertion takes place plays a vital role.

The assembly of all the cylindrical-shaped microneedle arrays used enables skin perforations by simply putting the instrument on the skin's surface and generating stress therein as well, which promotes needles puncturing through the corresponding advanced needle. This appears to be favorable because less force is often required than when using flat microneedle assemblies [40,41]. Using a rotary drilling method, microneedles were inserted into the layer of skin at exact depths inside the dermis or epidermis. By partly retracting microneedles after placing them, the flow rate into the skin was significantly boosted for the administration of microscopic dye molecules, insulin, polymer particles, and cells [42].

Some of these rollers, such as home dermarollers and beauty mice, are also made for personal use. Many businesses, like Hansderma and White Lotus, have marketed goods with comparable uses. With the introduction of DermastampsTM, tiny replicas of the Dermaroller on the UK market, PCI technology has now advanced. These are specially made to fit into limited, tiny spaces where the dermaroller finds it challenging to obtain the best stimulation, including the upper portion of the lip. The device is thought to work best on secluded scars and wrinkles.

6.2 Dermapen

An improved microneedling device called the Dermapen has been introduced to address the problems of inconsistent application of pressure by

doctors and patients and the ensuing microneedle penetration obtained. Manufacturers refer to it as a fractional microneedle device, loaded with a spring that performs "fractional mechanical resurfacing" and has an adjustment ring that allows for changing the heights of the microneedle. It works by applying an oscillating stamp-like action to the skin with an electrical-driven pen, causing the skin to generate a number of tiny channels. The producers are looking at treatments for photoaging, burn scarring, and acne scarring. More commercial solutions based on these microneedle technologies are currently in development as a result of the strong interest in them [17].

7 Advantages of microneedling

There are many benefits of microneedling as the method stimulates collagen development without removing the epidermis. For us to regenerate facial skin and actually seem youthful, we need a flawless epidermis with original dermal papillae, original color, good moisture, and regular resilience. A procedure that enables us to safely treat any area of skin is necessary for real regeneration because we also need to treat other bodily parts. The fibroblasts' encouragement of fresh collagen and elastin synthesis is crucial in this situation. These structural proteins, which are responsible for most of the skin's physical characteristics, gradually degrade with age as fewer new ones are created.

Skin loosening causes surface fine lines and gravity folds. In reality, although collagen and elastin synthesis are high during infancy, it tends to cease around adolescence and then gradually decreases as you grow older. The process of skin aging starts to manifest when the ratio of protein synthesis to degradation shifts negatively [43]. Basically, the ancient collagen structures that are the bridge between the scar with the top dermis are broken down by the needles. The related damage triggers the typical wound-healing inflammatory cascade, and new collagen is deposited beneath the epidermis to replace the scar collagen that has broken down. But the epidermis remains intact throughout this process [44].

The recovery period and danger of side effects are both reduced because of this less invasive technique, unlike the traditional method which involved the risk of infection, hypersensitivity, lumps, discoloration, etc. [45]. The skin thickens and becomes more resistant, unlike ablative procedures, where the cicatricial tissue is more vulnerable to photodamage. Fibrous tissue that replaces lost tissue and connects continuity solutions makes up cicatricial tissue. It is recommended for all skin kinds and colors, and it can even be used

in places where there are fewer sebaceous glands. It is also less expensive than procedures requiring technology with large investment requirements [46].

In the case of cosmeceuticals, microneedling is the primary method used to induce percutaneous collagen in antiaging treatments. One benefit of percutaneous collagen induction is that it doesn't harm the skin. Histology has demonstrated that the skin cannot be distinguished from regular skin, and that dermal papillae may be more visible in the epidermis. The skin thickens, accumulating more than 400% more collagen and considerably more elastin. The entire body can be treated in this method.

The recovery period is brief. It costs less and results in better skin than laser resurfacing. Darker-skinned individuals may undergo the procedure without concern about hyperpigmentation. Sun sensitivity does not develop in the skin. This can be performed on persons with very thin skin or those who have undergone laser resurfacing. Using a novel tool created especially for the process, the technique is simple to grasp and does not always require the assistance of a dermatologist or plastic surgeon. In some cases, Percutaneous Collagen Induction can be performed while employing topical anesthesia [47].

7.1 Vaccine and drug delivery

A hypodermic needle is used to inject the majority of biotherapeutics and vaccinations. Injecting practically any kind of chemical into the body is a simple, quick, and straightforward process. However, because hypodermic needles are difficult for individuals to use on their own, they are normally used exclusively by people who have received special instruction in suitable injection techniques, safe needle recycling, and additional issues at home or in clinics. In addition, the pain and needle anxiety that many patients experience hinder patient compliance. Another significant worry is the spread of bloodborne diseases caused by reused needles, particularly in developing nations.

The majority of these issues are resolved by oral distribution; however, many medications cannot be administered via this route because of inadequate absorption and drug degradation in the liver and gastrointestinal system. Although several delivery methods have been looked into, none have been found to be as effective overall as it is the same as a needle injection. Rather than eliminating needles, it was proposed to micronize them to use their powerful delivery characteristics while improving the patient's safety and compliance. A microscale tool, such as a microneedle, needs to

be big enough to distribute almost any pharmaceutical or minuscule particle composition while staying sufficiently tiny to avoid discomfort, anxiety, and require specific expertise to administer.

A microneedle additionally allows for precise tissue localization, allowing entry into places including the skin, the suprachoroidal region of the eye, and the cell nucleus. To present, the majority of microneedle applications have focused on skin administration of medications and vaccinations. The barrier qualities of the stratum corneum, the topmost layer of skin, place restrictions on conventional transdermal delivery. Skin permeability has been examined using a variety of chemical, biological, and physical techniques. To transport biotherapeutics and vaccinations across the skin, chemical and biochemical approaches do not seem to be particularly effective.

Although physical techniques show greater potential for macromolecule distribution, they usually necessitate the deployment of modern equipment that is quite big, costly, and labor intensive to operators. Microneedles, on the other hand, may be made at a very cheap cost as a patch that patients are able to use for biomacromolecule distribution, as discussed further in this study. Microneedle-based vaccine administration that specifically targets skin cells that present antigens is also of interest. Microneedles have also been researched for other uses. Recent research has focused on drug administration to the eye, particularly through the suprachoroidal region. Microneedles have been used in laboratories to carry chemicals into cells and their nucleus, among other things, as an extension of micropipette procedures [48].

Studies that looked into the utilization of the MN system in the development of immune therapies for cancer, skin disorders, allergic reactions, and other complicated medical illnesses resulted in significant advances. Any type of distribution platform must constantly take price into account. For a variety of reasons, administering vaccines through hypodermic injection has long been regarded as costly.

Among them are the requirements for cleanrooms, freeze-drying, autoclaving, intricate and proficient routes of distribution, cold chain supply networks, needles, syringes, water for injection to reconstitute the freeze-dried powder, and the requirement for instructed medical personnel to use the injection. In contrast, due to several important benefits, microneedle aided vaccination delivery has become a more affordable method than traditional hypodermic injections. Microneedle patches can be applied without clean rooms, cold chains, or medical professionals with the appropriate

training. They further allow for reduced dose frequencies and dose-sparing, in addition to the use of polymers, plastics, metals, and other materials. Together, these elements significantly reduce the overall cost.

Based on a considerable number of medical and premedical studies that include the COVID-19 vaccine, microneedles-directed vaccine administration could provide higher stability, safety, effectiveness, and cold chain free distribution as compared to standard vaccine formulations and delivery. This innovative vaccine delivery system's successful commercialization will prove groundbreaking and potentially save numerous lives throughout the world, particularly in resource-constrained nations where mortality rates caused by different transmissible diseases are excessively high [49].

7.2 Point-of-care diagnostics

Recent outbreaks of infectious diseases, such as COVID-19 and Ebola, have brought attention to the importance of quick and precise diagnosis to start treatment and stop transmission. The effectiveness of biological samples and prompt analysis are essential components of effective diagnostic techniques. The substantial bottleneck caused by the need for specialized equipment and qualified personnel in current diagnostic approaches, however, makes them invasive and obtrusive. Furthermore, it may enhance disease transmission because centralized test facilities are difficult to obtain.

Those who are self-administrable point-of-care (PoC) microneedle diagnostic tools might be an effective way to address these issues. These tiny needle arrays can enable (almost) real-time diagnostics by minimally invasively detecting biomarkers in/from the skin. Although very similar techniques are already widely utilized in various sectors and are usually readily suitable for infectious disease detection, few microneedle devices are designed expressly for the diagnosis of transmissible illnesses. These comprise analyte-capture microneedles, biofluid extraction microneedles, and microneedle sensors, alone or in combination. Analytes can be sampled or detected through both cutaneous interstitial fluid and blood. The techniques used for identifying infectious illnesses are still in the earliest stages of research, yet there's a huge amount of room for advancement going forward.

Although solid, hollow, hydrogel, and porous microneedles can all be used to extract ISF (intestinal fluid) and blood, empty microneedle extracting techniques have advanced the most. These devices allow skin biofluids to be collected onto a chip and analyzed in microfluidic chambers, or the biofluids gathered can be transmitted outside the device for examination using

common commercial tests. To examine if there are some concerned analytes present, microneedle-based biofluid extraction devices typically work best when in combination with downstream analytical techniques. These methods range from traditional lab-based detection techniques, such as microscopy, cell culture, enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction, high-performance liquid chromatography, and proteomic analysis, alternate analyte detecting methods [50].

The point-of-care diagnostic gadget of the future must be non-invasive. With trustworthy data, tissue damage must be kept to a minimum while patient comfort is maintained Microfluidic technology has the potential to greatly enhance ISF testing and diagnosis in three distinct domains: (i) no pain microneedles for quickly gathering sizable quantities of interstitial fluid, (ii) wearable Micro needles integrated with biosensors for better in situ and continuous interstitial fluid collection and detection, and (iii) an improved in vitro epidermis model for ISF circulation [51].

The manufacture of hollow microneedles in silicon using a novel design was presented. A wide range of needle shapes is possible because of the fabrication process, which entails a series of anisotropic wet etching and conformal thin film deposition stages. Based on preliminary studies, microneedles have the durability and precision needed in transdermal usage. To assess the potential medical applications, several animal studies were conducted. Finally, a capillary electrophoresis chip was equipped with a microneedle array that served as a blood sample [52].

The current generation of wristbands and smartphones can readily monitor activity and vital signs, but a new wave of wearable electronics is rapidly coming that will enable individuals to follow wellness indicators at the level of molecular. The transdermal sensors are microneedle-based and uniquely designed to serve a significant part in combining the useful benefits of cutaneous ISF as a major source of clinical variables with painless skin puncturing to enable the collecting of actual time data for diagnosis in this emerging era of wearables.

While early attempts at microneedle sensing focused on dermal interstitial fluid extraction in conjunction with either off-chip instrumentation or on-chip analysis, recent progress has focused on mounting biosensors with electrochemical sensors on the outermost layer of microneedles to enable govern continuing chemical measurement data. With this regard, significant progress has lately been achieved in the use of microneedle-based technologies for the simultaneous combinatorial identification of critical chemical indicators as well as actual time evaluation of a variety of electrolytes, metabolites, and medicines [50].

8 Microneedle design and structure

While creating microneedles for skin penetration, the following considerations should be taken into account: (a) geometrical characteristics, including diameter, length, tip size, and form, (b) physico-morphological characteristics like hollow, solid, conical, side-opened, beveled tip (c) selection of the material, (d) viability of manufacture, (e) use, (f) array design (g) density, (h) total microneedles, and (i) the state of the surface layer (for example, hydrophobicity). All microneedles could be classified as out-of-plane or in-plane, with their layout varying according to the fabrication and application process. Out-of-plane microneedles possess a longitudinal center perpendicular to the substrate's surface, whereas in-plane microneedles possess the shaft's longitudinal center parallel to it [53].

Microneedle design and geometry, such as apex radius, shape, and foot width, length, dimension ratio, and so on, are critical in defining both mechanical qualities and overall performance. To generate optimal microneedles that are mechanically powerful enough to puncture the outermost skin, biocompatible, suitable for transporting numerous active therapeutic components, and capable of sustained or controlled drug deliverance, the correct material and procedures must be carefully chosen. The geometry of Microneedles, which is their most important component, makes for easy insertion into the skin.

This is significant since human skin tends to inhibit microneedle insertion or potentially cause microneedle rupture due to its elasticity and robustness. This is evident in microneedles, which are made of flimsy materials and have blunt points. As a result, Microneedle geometry must be delicate and precise to deliver drugs effectively. The capability of the microneedle to rupture the skin with minimal force depends on how sharp the tip of the microneedle is, and the opposite is true for MNs with tips that have a greater diameter. Suitable MNs for clinical use must be mechanically robust, biocompatible, and have a high potential for drug cargo loading [54].

As it was previously discussed the four kinds (solid, hollow, dissolving, and coated) microneedles are differently structured and classified based on their application. Depending on the component portions or design of the patch, microneedles could be manufactured from a range of materials, like metal and polymer. Microneedle materials should, in general, be adequate in strength to pierce the skin. It must also decay or disintegrate in the human body without causing toxicity. During the production, storage, and transportation of the microneedle patches, drug and matrix compatibility is essential.

Silicon is frequently utilized to make solid and coated microneedles because it possesses the mechanical strength necessary for skin implantation. Metal materials have a strong tensile and mechanical strength, allowing them to travel easily via the skin. Coated, solid, and hollow microneedles are made using them. Microneedles tend to be made of stainless steel and titanium (Ti).

Stainless steel is the most often metal used to make microneedles, however, it corrodes faster than titanium alloy. Titanium alloys cost more than stainless steel however offer higher mechanical durability. Microneedle polymers must be biocompatible, water-soluble, and robust enough to pierce the skin. Glass microneedles, which are generally hollow, are made via a micropipette puller or wet etching. It has enough strength to get into the skin, making the tapered shape easy to process.

Sterilization is relatively easy since the material is stable at elevated pressures and temperatures and is biocompatible. It does, however, break readily; especially if the sharp point of the microneedle comes off and remains in the cutaneous tissues, it can result in tumors or inflammation. Studies have looked into the usage of ceramic materials including calcium phosphate, calcium sulfate, and alumina in the creation of microneedles since they are biocompatible and have adequate mechanical strength [55].

For compounds that are not easily absorbed topically, microneedles offer a simpler approach to transdermal medication delivery. The main goal in the manufacture of microneedles is to achieve reproducibility and uniformity of the needle shape at a resolution micron scale to make it simpler for the needles to penetrate the skin. The use of conventional lithography in microfabrication techniques has great promise in this area. The 3D conical shape and high aspect ratio structures of the microneedles, whose height ranges from 0.5 to 3 mm, have been proven to make the manufacture of microneedles extremely difficult. Molding techniques are most frequently used to create microneedles, and in these techniques, intricate microfabrication operations are used in the cleanroom to prepare the molds. Pectin, a natural polymer, as well as PVP and PVA, semisynthetic or synthetic polymers, are all manufactured by micromolding.

Several methods are used to uniformly put liquid polymeric solutions over the mold, subsequently followed by the removal of air gaps. A vacuum can be used to remove air gaps, which can then be dried in an oven before being removed from the mold completely. The centrifugation or vacuuming phase required in micromolding processes can make it difficult to manufacture microneedles continuously. This issue can be rectified by atomizing the polymer solution and spraying it into the molds.

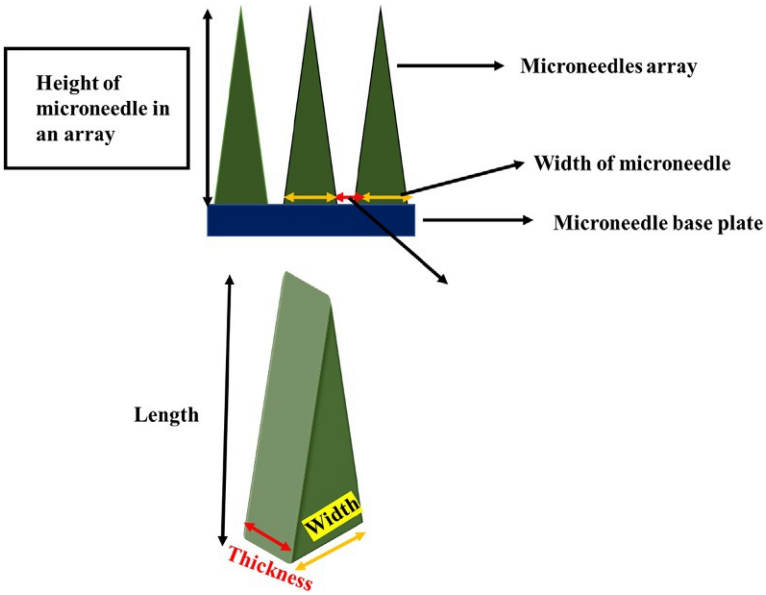

Fig. 3 Geometrical parameters in a microneedle array.

Experimentation proved that aqueous solution atomization may effectively cover the whole mold surface and not create voids. The wet etching technique is another fabrication process. The substrate is immersed in a solution of chemicals during this method to help in the removal of superfluous material. The wet etching procedure can be carried on either isotropically or anisotropically—at the same pace or at a different rate. It is employed to create silicon and metallic arrays. In the laser cutting process, metallic sheets are sliced into the shape of tiny needles using an infrared laser.

The geometry of the microneedle is designed using AutoCAD software. The metal sheet is subsequently removed by the laser beam in accordance with the design, creating in-plane microneedles. The manufactured microneedles are cleaned and bent perpendicularly. The tips are then electropolished to adjust them. Metal microneedles are produced using this technique. Stainless steel is the material that is used most commonly [56]. The geometrical parameters of the microneedle array can be described in Fig. 3.

9 Conclusion and future perspective

The demand for microneedles has only increased over the past one or two decades. From the rapid inventions in this arena, somebody can easily predict that much research has been happening in this field. Its ability to

intervene in the protective and rigid layer of skin has been the start of all these inventions. As a painless drug delivery major, microneedles have been an amazing invention in the healthcare sector. Its mechanism includes percutaneous collagen induction by initiating the body's natural wound-healing response. Consecutively, the various kinds of microneedles and their fabrication method helped treat minor diseases like influenza to serious chronic diseases like cancer and diabetes. This alternative drug delivery method provides the privilege of injecting drugs into patients' bodies by themselves with the help of microneedle patches. Microneedles also resolve the need for rapid diagnostics as they help analyze the interstitial fluid of the human body. After health care, scientists took a dig into the skincare field. Dermarollers and dermapens made the user experience very convenient in cosmeceutical treatments. Microneedling treats acne, scars, and hair loss in the dermatology sector. Still, much attention is given to antiaging treatments as it is a highly convenient method with minimum side effects compared to traditional surgical treatment. The structure and design play a significant role in serving its purpose. Currently, much research is going on in this field, which surely would invite more quick and efficient methods of various cosmeceuticals deliveries.

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CHAPTER 11

Designing, characterization, and efficacy of microneedle patches in management and treatment of obesity

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1 Introduction

Due to their effectiveness and lack of discomfort, microneedles are regarded as valuable medication delivery systems. Both independent and interconnected systems constituting complex microfluidic systems may be created using microneedles [1]. Despite the fact that the concept of microneedles was first proposed in the 1970s, it wasn't until the 1990s that it was experimentally proven, thanks to the microelectronics industry's delivery of the necessary equipment for microfabrication. In 1998, the first studies of transdermal drug delivery were published. Since then, there has been a sharp rise in the development and deployment of microneedles for the delivery of drugs and other pharmaceutical uses [2]. In many therapeutic situations, the transdermal route is employed to get around the significant drawbacks of oral medication delivery techniques. Recent advancements in this field demonstrate the value of microneedle array (MN) systems for transdermal medication delivery of substances that cannot diffuse through the skin naturally or with the assistance of an external source. It has been shown that MN is an efficient method for delivering compounds with high molecular masses. Also, it is intended to prevent pain-related problems when receiving an injection using a hypodermic needle [3].

Obesity is a disease that results from an excessive accumulation of fat. It is a health problem that makes it more likely for people to acquire illnesses such as cardiovascular disease, diabetes, hypertension, and various cancers. For a variety of reasons, some people find it difficult to lose weight [4]. Obesity is the result of a complex interaction of environmental, genetic,

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and physiological factors as well as nutritional, physical activity, and lifestyle factors. The good news is that losing any amount of weight can aid in reducing or avoiding the health problems associated with being overweight. You can achieve your weight loss objectives by altering your eating patterns, increasing your physical activity, and changing your behavior. Prescription medications and weight-loss surgical procedures are further treatments for obesity [5].

BMI calculation

The body mass index (BMI) can be used to estimate the percentage of body fat. The body mass index (BMI) is calculated by dividing the kilogram weight by the square of the height in meters.

$$\mathrm{BMI} = \mathrm{weight}/\mathrm{height}^2$$

BMI	Weight status
Below 18.5	Underweight
18.5–24.9	Normal
25.0–29.9	Overweight
30.0 and higher	Obesity

Asians with a body mass index (BMI) of 23 or higher may have an increased risk of developing health problems [6].

Excess body fat (white adipose tissue—WAT) is the notorious risk factor for many health problems, such as diabetes, hypertension, coronary heart diseases, stroke, and some cancers (e.g., colorectal cancer). According to the World Health Organization, at least 2.8 million people die worldwide each year as a result of being obese, and an estimated 35.8 million (2.3%) of global disability-adjusted life years are caused by obesity [6]. There is thus a growing demand for obesity management and treatment. Skin patches are already used in cosmetics and vaccine delivery. To address the urgent and unmet need, this chapter reports a new strategy using disposable transdermal patches equipped with detachable polymeric microneedle (MN) array of varying degradation profile, for localized delivery of antiobesity compounds directly targeting on the subcutaneous WAT. At present, the bioavailability of nutraceuticals at adipose tissue in obese population is very poor. The micro- and nanoformulations developed under this project will be highly bioavailable with targeted delivery at the sites of adipose deposition through

microneedle transdermal patches. This technology will help the obese population to spend the excess energy by converting WAT to BAT without any physical exercise and diet restriction.

1.1 Etiology

Regardless of how a person behaves or how many calories they burn off through daily activities and exercise, obesity develops when they consume more calories than they burn off. Your body will retain the additional calories as fat. Since fast food and sugary drinks make up a major portion of most people's diets, they typically contain too many calories [7]. Overweight individuals may eat more than they require, feel hungry more frequently, or overeat as a reaction of negative emotions such as stress and worry. Due to the fact that jobs are not as physically demanding as they formerly were, people in many nations do not normally burn as many calories while working. The quantity of calories consumed during even the most routine daily activities has decreased, thanks to conveniences such as remote controls, escalators, online shopping, and drive-through banks [8].

1.2 Risk elements

i. Pregnancy: Pregnancy-related weight gain is typical. Some women may find it challenging to lose the extra weight after giving birth. This weight gain may increase the number of obese women [9].

ii. Giving up smoking: Gaining weight is a typical negative side effect of stopping smoking. Some individuals may even gain enough weight as a result to qualify as obese. When someone is quitting smoking, they typically start eating instead. Long-term health benefits of quitting smoking outweigh those of continuing to smoke, nevertheless. How to prevent putting on weight after you stop smoking might be discussed with your doctor [10].

iii. Lack of sleep: Sleep deprivation or excessive sleep duration might cause hormonal changes that increase hunger. You could look for foods that are heavy in calories and carbohydrates, which could lead to weight gain [11].

iv. Stress: Weight gain can result from a number of environmental factors that have an effect on mental health. Many people turn to comforting high-calorie snacks when under stress.

v. Microbiome: Your capacity to gain or lose weight may be impacted by how you eat, which may change the makeup of the bacteria in your intestines [12].

1.3 Complications

Obese people are more likely to experience a number of serious health problems, including strokes and cardiovascular disease. Obesity, high blood pressure, and high cholesterol are all risk factors for heart disease and stroke [13].

Type 2 diabetes: Obesity may impair the body's capacity to use insulin to control blood sugar. As a result, developing diabetes and insulin resistance is more likely.

A few types of cancers: Obesity has the potential to make cancers of the cervix, endometrium, ovary, uterus, breast, colon, rectum, esophagus, liver, gallbladder, pancreas, kidney, and prostate worse [14].

Food digestion issues: Being overweight increases the risk of developing heartburn, gallstones, and liver problems.

Apnea when sleeping: Obese persons are more likely to have sleep apnea, a potentially fatal condition in which a person's breathing regularly stops and starts during the course of the night.

Osteoarthritis: The weight-bearing joints of the body are also put under additional stress by the inflammation brought on by obesity. One issue that can arise from these reasons is osteoarthritis [15].

1.4 Treatment

i. Altering a person's diet: Losing weight requires calorie limitation as well as the adoption of healthier eating practices. Although losing weight quickly at first is alluring, the healthiest and most long-lasting strategy is to lose weight gradually. No diet is "ideal" for weight loss. Choose one that offers healthy solutions that you think will benefit you [16].

ii. Making healthy changes to one's diet can aid in weight loss by cutting back on calories. The main weight-loss method is reducing calorie intake. The first step is to look at your typical eating and drinking habits to figure out how many calories you consume everyday and where you can cut back. Dietary calorie needs for weight loss vary from person to person, but typically lie between 1200 and 1500 cal for women and 1500 and 1800 cal for males [17].

iii. Less hunger and satiety: Fats, processed foods, desserts, and sweets have the highest calories per gram of weight. On the other hand, fruits and vegetables provide more volume for the same amount of calories. You can eat less while still enjoying your meal and improve your sense of fullness by sating your hunger with larger portions of lower-calorie foods [18].

iv. Making wiser food and activity choices: The majority of your diet should consist of plant-based foods such as fruits, vegetables, and whole grains if you want to improve your health. Add lean meats and other low-fat protein sources to your diet, such as beans, lentils, and soy. If you like fish, try to eat it at least twice a week. Limit your sugar and salt intake. When choosing healthy fats for your meager daily requirement of fats, consider olive, canola, and almond oils.

v. Restricting your consumption of certain foods: On these kinds of diets, foods high in fat or carbohydrates are frequently forbidden. Find out what diet plans your doctor suggests from them. A guaranteed way to consume more calories than you intended is to consume sugary beverages. Reduce your intake of or completely stop drinking these beverages if you are attempting to lose weight [19].

vi. Meal replacement products: These diets advise consuming low-calorie drinks, meal bars, and healthy snacks in addition to a third, low-fat, calorie-restricted meal as well as replacing one or two meals each day. On such a diet, weight loss is possible, at least momentarily. However, adhering to these diets generally will not teach you anything about making long-lasting lifestyle changes. You must be dedicated to the eating regimen if you want to keep the weight off.

vii. Exercise: People who are obese should exercise for at least 150 min a week at a moderate level to prevent weight gain or maintain even a little amount of weight loss. You should gradually boost the intensity of your workouts as your fitness and endurance improve.

viii. Changes in behavior: A behavior modification program can help to lose weight and keep it off. You could examine your current routines to see what stresses or situations can be to blame for your weight increase [20].

ix. Counseling: Speaking with a mental health professional can be helpful for those with eating-related emotional and behavioral disorders. Therapy can help you control your anxiety, which can manifest itself as overeating. You may learn how to manage emotional eating, create a food and exercise log, and figure out what triggers your appetite. In the realm of therapy, both individual and group sessions are allowed.

2 Obesity management by other therapies

Medication for weight loss should not take the place of healthy lifestyle modifications such as eating right and exercising frequently. Before giving you any medication, your doctor will consider your medical history and

any possible drug responses. Treatments for obesity that have received FDA approval include orlistat (Alli, Xenical), bupropion-naltrexone (Contrave), liraglutide (Saxenda), and phentermine-topiramate (Qsymia). Drugs for weight loss do not work for everyone, and they lose some of their potency with time. You risk gaining back all the weight you lost and then some if you use a weight-loss supplement and then stop taking it [21].

i. Endoscopy is used to manage obesity; this type of treatment does not require incisions. A flexible tube or other equipment is inserted into the stomach through the mouth of the patient after they have fallen asleep. The endoscopic sleeve gastroplasty procedure is frequent. By sewing the stomach shut, this therapy reduces the stomach's capacity. The ordinary person can lose weight over time by gradually reducing their food and beverage intake [22].

ii. Losing weight by using an intragastric balloon: A small balloon is injected into the stomach using this method. You can make your stomach believe it is fuller than it actually is by filling the balloon with water and eat less without getting hungry.

iii. Having weight-loss surgery: The amount of food a patient can eat or the number of calories they take in are both limited by weight-loss surgery, also referred to as bariatric surgery. However, this could also result in a lack of vital vitamins and nutrients.

iv. For people trying to lose weight, adjustable gastric bands are a popular surgical procedure. Using an inflatable band, the stomach is separated into two pouches in this procedure. The surgeon tightens the band so that it resembles a belt to form a narrow path between the two pouches. The band is designed to be securely fixed in place and prevents the aperture from expanding.

v. Gastric bypass surgery. Gastric bypass (Roux-en-Y) involves the creation of a small pouch at the top of the stomach. Next, an incision is made immediately below the stomach properly to link the new pouch to the small intestine. This portion of the intestine bypasses the stomach totally and takes food and liquids directly from the pouch.

vi. Gastric sleeve surgery. The patient's stomach will be smaller and able to hold less food as a result of this procedure. This technique is significantly easier than gastric bypass.

Another treatment option for obesity is hydrogels. You will feel fuller more quickly, thanks to the tiny particles inside these capsules, which absorb water and swell up in the stomach. The pills should be swallowed and passed via the stool before every meal. Another therapeutic option is the blockage of

vagus nerve activation [23]. The brain and the vagus nerve in the abdomen connect to let you know when you are full or hungry. This nerve receives sporadic electrical pulses from an implanted device. Another way to achieve your weight loss goal is by setting reasonable goals. When you need to lose a lot of weight, you can try to do so too soon or by establishing unattainable goals. Do not subject yourself to needless pressure. On a daily or weekly basis, plan out your exercise and weight-loss program. Instead of making drastic changes to your diet that you will not be able to keep, modify it gradually [13].

Continuity of treatment as directed is also important. It could be difficult to break long-standing habits. It is crucial to be open and honest with your doctor, therapist, or other healthcare experts if your fitness or diet goals start to slip. You can pool your resources to come up with original plans. You can request aid and invite your loved ones to support you in your weight-loss efforts. Instead of spending time with those who will hold you back, surround yourself with those who will support and encourage you. Make sure they understand how important it is for your health that you lose weight. You can also get support from a group that helps people lose weight. Maintaining a diary or record is also useful. Keep a daily food and activity journal. Monitoring your diet and exercise routine could help you stay on track with your health objectives. You can determine what behaviors are advancing you and what behaviors are restraining you. You may monitor your blood pressure, cholesterol, and general fitness with your health journal [21].

3 Polyphenols in obesity management and treatment

Plant polyphenols, a large class of naturally occurring antioxidants, are worthy contenders for providing substantial protection against cancer and cardiovascular disease. Polyphenols' antioxidant properties have been thoroughly studied, and it is now known that their mechanisms of action extend beyond just lowering oxidative stress. Polyphenols, according to new research, are useful in the treatment of obesity via a mechanism involving their antioxidative characteristics and the elimination of free radicals [24]. Polyphenols boost the transport of unsaturated FA, which increases the gene expression of these enzymes linked with thermogenesis, adipogenesis, or FA oxidation. This reduces TC and TG levels in the blood as well as fat deposition. *Crataegus pinnatifida* Bunge leaf contains the flavone present in hawthorn leaves. High-fat diet (HFD) obese male lavages were given to

Sprague-Dawley rats at doses of 12, 6, and 3 mg/kg/d for 18 days. According to experimental research, the flavonoid intervention may successfully lower serum and liver TC and TG levels. Furthermore, fat formation in adipose tissue was limited because the level of lipoprotein lipase (LPL) in muscle tissue rose while it decreased in adipose tissue. Furthermore, some polyphenols can reduce fat storage by modulating the activity of proteins involved in adipocyte formation [3]. The majority of resveratrol is found in seed coats, where it can increase the expression of the protein silent information regulator 1 while decreasing the expression of the proteins PPAR and CCAAT/enhancer binding (C/EBP) in fat cells to prevent preadipocyte proliferation and differentiation, lowering fat synthesis.

Several active phenolic and flavonoid compounds, according to previous descriptions, may be able to both prevent and treat obesity. Weight loss, improved blood lipids, and IR management are all apparent instances of how flavonoids influence pharmacokinetics. Polyphenols have consistently piqued the pharmaceutical industry's interest as bioactive natural chemicals for the treatment of obesity. Several studies document that food bioactives (caffeine, curcumin, piperine, and capsaicin), natural ingredients, have a therapeutic activity and possess an antiobesity activity without undesirable effects [9,13,25,26], which has been proven to exert terrific antiobesity activity, particularly with the aid of using, promoting the white adipose browning and suppressing lipogenesis (Fig. 1).

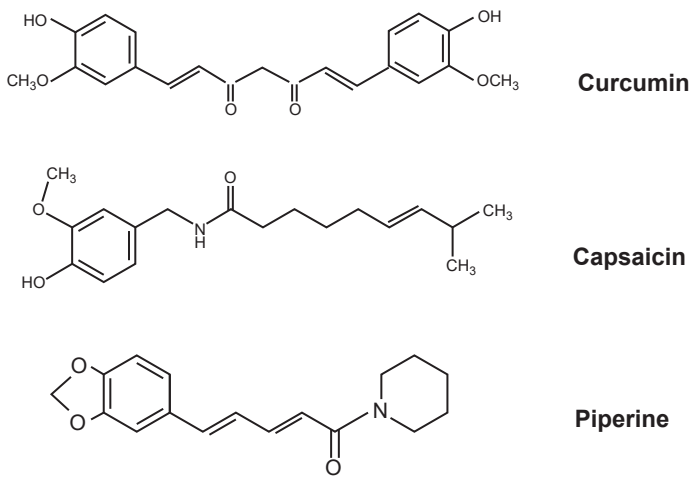

Fig. 1 Chemical structure of curcumin (turmeric), capsaicin (red pepper), and piperine (black pepper).

3.1 Curcumin

The spice turmeric has drawn a considerable lot of interest from the scientific, medical, and culinary worlds. Turmeric (*Curcuma longa*), a herbaceous perennial with rhizomes, is a member of the ginger family [27]. Although curcumin, a component of turmeric, has been used medicinally for thousands of years, the particular mechanism(s) of action and the bioactive components have only recently been identified. Curcumin, also known as diferuloylmethane, is the main natural polyphenol found in the rhizomes of *C. longa* (turmeric) and other *Curcuma* species. It is also known as 1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione. *C. longa* has been used as medicine for centuries in Asian countries due to its antioxidant, antiinflammatory, antimutagenic, antimicrobial, and anticancer attributes, antimutagenic and antimicrobial properties [28].

The multiple health benefits of the polyphenol curcumin have been supported by evidence that it targets a wide range of signaling molecules and also exhibits activity at the cellular level. The treatment of inflammatory and degenerative eye problems, metabolic syndrome, pain, and inflammatory conditions has been shown to benefit from it [29]. Additionally, it has been shown to be beneficial for the kidneys. Although consuming curcumin supplements appears to have a myriad of therapeutic benefits, the majority of these benefits are brought on by its antiinflammatory and antioxidant capabilities. Although curcumin has been proven to have antiinflammatory and antioxidant characteristics, one of the primary problems with taking curcumin alone is that it has a poor bioavailability, which appears to be mostly brought on by poor absorption, quick metabolism, and rapid elimination [30]. A number of drugs have been tested to improve the bioavailability of curcumin by addressing these various mechanisms. Most of them were developed to inhibit the metabolic pathway of curcumin to increase its bioavailability. Black pepper, for example, contains piperine, a renowned bioavailability booster, which has been associated to a 2000% increase in curcumin bioavailability. Therefore, increasing bioavailability by adding compounds such as piperine and creating a curcumin complex seem to be solutions to the low bioavailability issue [31].

Curcumin's ability to lower systemic inflammation has implications beyond arthritis because systemic inflammation has been connected to a variety of disorders affecting several systems. One such condition is metabolic syndrome (MetS), an illness characterized by insulin resistance,

hyperglycemia, hypertension, low levels of HDL-C and high levels of LDL-C, elevated triglyceride levels, and obesity, particularly, visceral obesity. Curcumin has been shown to attenuate many aspects of MetS by increasing insulin sensitivity, decreasing adipogenesis, and lowering hypertension, inflammation, and oxidative stress [3]. Furthermore, there is evidence that curcuminoids change gene expression and the activity of enzymes involved in lipoprotein metabolism, lowering plasma triglycerides and cholesterol while increasing HDL-C levels.

3.2 Piperine

Piperine is an amide alkaloid with many beneficial effects, including those on the immune system, inflammation, blood pressure, the liver, the nervous system, and cancer prevention. It also has impacts on fertility and increases bioavailability. The bioavailability of many medications is affected by piperine, and it can also affect the enzymes responsible for their metabolism and the health of the digestive tract [32]. This review looks at the role piperine plays in the prevention, management, and treatment of a wide range of diseases and disorders by analyzing the most recent clinical and preclinical data, nanoformulations, extraction methods, structure-activity relationships, molecular docking, bioavailability enhancement of phytochemicals and medications, and brain penetration properties. Diabetes, obesity, arthritis, multiple myeloma, oral cancer, breast cancer, metabolic syndrome, hypertension, Parkinson's disease, Alzheimer's disease, cerebral stroke, cardiovascular disease, kidney disease, viral disorders, and rhinopharyngitis are only few of the conditions that piperine can treat. Cell cycle proteins, antiapoptotic proteins, P-glycoprotein, cytochrome P450 3A4, MRP1, TRPV1, nuclear factor-B, c-Fos, cAMP response element-binding protein, activation transcription factor-2, and peroxisome proliferator-activated receptor-gamma are just some of the signaling molecules that can be modulated by piperine [33].

When piperine is used to treat metabolic issues, a bioavailability enhancer is frequently used. The pathology of obesity, the most serious medical condition, is caused by a rise in energy consumption, which in turn causes adiposity and fat cell proliferation. Type 2 diabetes, coronary heart disease, and stroke are just some of the EFRMDs linked to being overweight or obese [34]. Feeding behavior can be affected by the neuropeptide

melanocortin-4 (MC-4) from the hypothalamus binding to its receptor. Increased MC-4 receptor activation leads to less hunger, higher energy expenditure, and improved insulin sensitivity. Studies suggest that the spice piperine, at a dose of 40 mg/kg, may help with fat metabolism by acting as an MC-4 agonist. Reversing plasma concentrations of adiponectin, insulin, and glucose, piperine (50 mg/kg bw) significantly enhances insulin signaling in HFD-induced hepatic steatosis [35]. Piperine (30 mg/kg) supplementation has been shown in another trial to reduce hypertension, plasma oxidative stress markers, and inflammation. As a successful adjunct medication for patients with metabolic issues, piperine (Bioperine) was coupled with curcuminoids in a randomized controlled trial to increase bioavailability.

3.3 Capsaicin

Chili peppers contain the phenolic compound capsaicin (8-methyl-*N*-vanillyl-6-nenamide), which gives chili peppers their characteristic flavor and pungency. Except for *Capsicum annuum*, all *Capsicum* genus members produce variable degrees of capsaicin, and humans have consumed all of them as spices for about 6000 years [25]. Capsaicin, along with salt, accounts for up to 1% of the mass of chili peppers and is the condiment that humans consume the most on average. Capsaicin is an intriguing molecule since it can elicit opposite experiences (pleasant and unpleasant) depending on the individual and chili pepper ingestion habits. Capsaicin's benefits extend far beyond flavor, and understanding how it affects plant health enhances appreciation of how it might assist human health [12].

The importance of thermogenesis and an increase in energy expenditure (EE) in the regulation of body weight by capsaicin has been highlighted in a number of studies. Among the putative biochemical processes underpinning capsaicin's regulatory function as EE, TRPV1 activation seems to be essential. Animals without TRPV1 and people with mutant (Val585Ile) TRPV1 both exhibit significantly diminished effects. Catecholamine release and subsequent SNS activation of -adrenoceptors via capsaicin-induced TRPV1 activation result in increased thermogenesis and EE [36]. Studies showing that -adrenergic blockers, including propranolol, impair thermogenesis support this mechanism. Brown adipose tissue (BAT), which is the main source of sympathetically triggered nonshivering thermogenesis,

has been proven to be the primary mediator of the thermogenic effect of capsaicin. Other effects appear to be relevant in capsaicin-induced increased EE, such as enhanced energy metabolism in skeletal muscle via TRPV1 activation and increased fat mobilization (triglyceride oxidation) in white adipose tissue (WAT) [11].

The process through which preadipocytes transform into adipocytes, known as adipogenesis, is in charge of closely controlling the quantity of adipose tissue. Capsaicin's modulatory effect on this pathway has been linked to a reduction in fat tissue. In a concentration-dependent way, capsaicin inhibits 3T3-L1 preadipocyte differentiation into adipocytes as well as the expression of adipocyte differentiation-related proteins PPAR, C/EBP, and leptin [37]. Similar effects are achieved by capsaicin, which stops bone marrow mesenchymal stem cells (BMSCs) from maturing into adipocytes. Capsaicin's impact on adipogenesis therefore extends beyond preadipocytes. The inhibitory effect of capsaicin on this pathway seems to be related to the activation of 5′ adenosine monophosphate-activated protein kinase (AMPK) and the generation of intracellular ROS. Anabolic pathways are blocked by activated AMPK, while catabolic pathways are encouraged. Therefore, AMPK activation prevents both cell growth and death [38].

This hypothesis is supported by the observation that capsaicin reduces preadipocyte development by preventing the S-phase of the cell cycle. Additionally, capsaicin lowers the proportion of BMSCs in the S phase and stops the cell cycle at G0-G1 [39]. Capsaicin activates caspase-3, Bax, and Bak, cleaves PARP, and downregulates Bcl-2 to cause apoptosis in preadipocytes. Additionally, capsaicin causes BMSC to undergo apoptosis by producing more reactive nitrogen species (RNS) and ROS [40].

4 Microneedle patches

In numerous therapeutic situations, the transdermal route is utilized to circumvent the substantial limitations associated with oral drug delivery methods. Recent developments in this field demonstrate the efficacy of microneedle array (MN) devices for transdermally administering drugs that cannot diffuse through the skin via passive diffusion or external source-assisted diffusion [10]. MN has been shown to be a viable method for transporting compounds with high molecular weights. In addition, it is designed to prevent pain-related complications during injections with hypodermic needle. Here, we discuss the current state, applications, and most recent

advancements of the microneedle array method for the delivery of molecules. Diverse approaches have been investigated to resolve the low potency of a medicinal agent.

To make microneedle-based treatment more accessible, especially in developing nations, future research will concentrate on developing methods to reduce loss during preparation and stacking, as well as enhancing the microneedle structures to ensure enhanced drug transfer [41]. Despite the fact that research on microneedle-based drug delivery systems is expanding each year, the issues associated with these systems must be addressed appropriately. As a consequence, microneedles could pass the clinical relevance test and contribute to their commercialization. Some microneedle-based medication delivery systems have begun Phase III clinical trials. However, the practical efficacy of microneedles in vaccination has yet to be established. As a consequence, efforts to administer the medication using a microneedle array have progressed and are now recognized as a viable alternative to hypodermic needles. This chapter focuses on how these systems can be utilized for transdermal drug delivery with potential therapeutic and safety benefits, as well as their most recent developments and clinical utility [42].

The microneedles are invisible to the human eye because of their height, which is less than 1 mm. This micron-sized needle creates the channel that enables the drug molecule to diffuse directly beneath the skin much more quickly by puncturing the stratum corneum, the skin layer that presents the highest barrier to the diffusion of the actives. MN was created in the 1970s by Martin S. Gerstel and Virgil A. Place. The report was released in the late 1990s, and it was used as a drug delivery method to directly provide a drug to the dermis layer by a percutaneous procedure and to produce a local and systemic pharmacological impact has been used to increase the bioavailability of protein-based medicines in addition to being painless [43]. MNs have made it possible to transport even very complex compounds, such as protein, vaccines, and peptides, transdermally. All varieties of MNs are finally used to achieve quick and efficient drug administration, whether they are solid, hollow, coated, or dissolving. The needle length must be adjusted in all varieties of microneedles to avoid piercing nerve endings as it penetrates the subcutaneous tissue (SC). There won't be any pain there if no nerve endings are damaged. In addition, these systems—as opposed to surgical implantation—can regulate the drug's release and ensure safe administration [44] (Fig. 2).

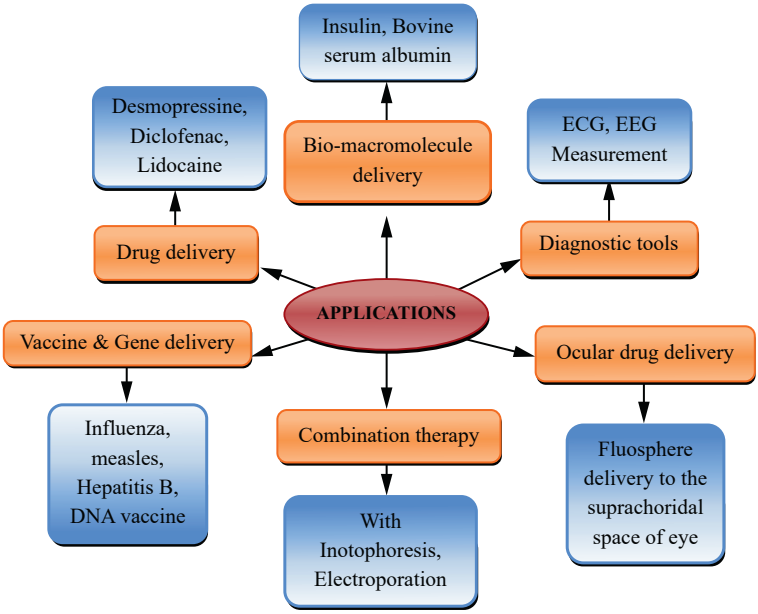

Fig. 2 Application of microneedle patches as transdermal drug delivery.

5 Conclusion

Microneedles provide numerous advantages over conventional needle/syringe as well as other delivery methods that may also be safe and effective. For example, a patch-based setup with several microneedle designs would facilitate simple vaccine observation and, most likely, patient self-administration. The small size of microneedle structures should make them easier to store and distribute to strategic spots. Solid microneedles are regarded as a striking platform for drug delivery due to their low manufacturing costs, and they can play an important part in the medical response to a viral pandemic. Solid microneedles are easier to make and stronger than hollow and dissolving microneedles. For biomedical applications, solid microneedles made of various materials such as glass, metals, silicon, and polymers have been reported; nevertheless, silicon has typically been employed as a substrate among other materials in the manufacture of microfluidic devices. Silicon is fragile and each time poses some health dangers. Biocompatibility is critical for health, which is why the trend is changing toward polymer materials. Most polymers, such as PDMS, PGA, PLA, and PMMA, are ideal for biomedical devices due to their high biocompatibility, low cost, ease of production, and remarkable chemical and mechanical properties. According to

the provided literature assessment, the authors conclude that microneedles, their commercialization, and biological applications are expanding on a daily basis and still require improvements and innovations.

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Declaration of conflict of interest

There is no conflict of interest to be declared by the authors.

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CHAPTER 12

Microneedles for various ocular diseases

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1 Introduction

Microtechnology, which has its origins in biomedicine, is currently being used in the pharmaceutical industry. The remarkable progress of modern manufacturing techniques has provided exciting possibilities for developing highly refined and accurate drug delivery systems. Consistently advancing pharmaceutical technology to new levels of dealing with various diseases, including those related to the eye, is a result of a big number of concepts and implemented projects, which is represented in a great number of scientific papers [1]. Many millions of individuals worldwide suffer from severe visual impairment or blindness as a direct result of eye disorders and traumas. The World Health Organization reports that at least 2.2 billion people have some form of visual impairment, either near- or far-sightedness [2]. Unaddressed refractive error, inflammation of the cornea, sclera, and iris, conjunctivitis, dry eye syndrome, allergies, retinopathy, age-related macular degeneration, cataract, glaucoma, central retinal vein occlusion, and diabetic macular edema are all common causes of moderate-to-severe vision loss [3]. Most of them can be totally or partially healed with the right diagnosis and treatment, but in the case of unsuitable or late treatments, permanent changes may emerge. Most of the aforementioned problems can only be treated with medication, either topically (on the eye's surface) or systemically (within the eye). Also, most pharmacological treatments require many applications over several weeks or months. The eye is a highly specialized sensory organ that has its own circulation and, as a result, has its own distinctive pharmacodynamic and pharmacokinetic characteristics [4]. It is composed of a variety of tissues, including smooth muscles, vascular and neuronal networks, connective tissue, and epithelia. Ophthalmic preparations are a clear substitute for

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oral forms, which have a number of drawbacks including slow absorption due to hepatic circulation and possible dietary interactions, a slow commencement of action, and systemic side effects [5]. Also, to get the desired concentration of medications in the orbit of the eyeball, greater doses must be used when administering through oral or intravenous routes. The most popular forms of ophthalmic preparations are eye drops, ointments, and gels, all of which deliver the drug in a dissolved or suspended form. They are applied on the eye, the conjunctival sac, or the eyelid by a medical professional or the patient themselves [6]. Their primary drawback is the inconvenient requirement for regular dosing. As a result, patients rarely adhere to the prescribed treatment plan and its efficacy declines. Drugs for the eyes can also be given as inserts. Most of them are either solid or semisolid shapes that are the right size and shape for the conjunctival sac [7]. They contain a reservoir holding the API that is covered by a matrix structure or film to control the rate of release. However, inserts are rarely utilized because of the difficulty in applying them, the potential for vision abnormalities, and the discomfort caused by the patients' perception of a foreign body in their eye. It is possible to classify inserts as either soluble (biodegradable/bio-erodible) or insoluble (therapeutic). Although many ophthalmic drugs can be safely and effectively administered using the noninvasive procedures, there are also a variety of invasive treatments available [8]. Subconjunctival and retrobulbar injections, depending on the formulation, might result in either an immediate or a sustained release. Retinal inflammation is the most frequently reported adverse effect following injection, although it is important to note that the drug's action cannot be controlled once it has started. The trend toward decreasing the size of needles became significant, leading to the introduction of microneedles in an effort to lessen tissue damage, membrane disruption, infection risk, speed of regeneration, and overall safety [9]. With today's ability to manufacture, needles shorter than 1 mm can be produced; however, nanometer-sized needles are projected to enter clinical use in the near future, radically altering the treatment of ocular illnesses. However, due to the extremely tiny size, microneedle injections call for highly trained specialists and cutting-edge technology. Ocular drug delivery microneedle systems/patches are being studied alongside single-microneedle technology [10,11]. At first, these arrays were thought to be a painless, noninvasive, and highly effective replacement for traditional methods of drug administration such as transdermal, intradermal, and percutaneous injections. Recent studies have shown that they are safe and effective for use on the eye's cornea and sclera. Numerous medicinal substances have been explored for delivery by microneedles in cutaneous

administration [2]. These treatments included antibiotics and antifungals used to treat local skin infections, as well as NSAIDs, antihypertensives, and lipid-lowering drugs that influenced the body as a whole [12]. To add to this, microneedles (MNs) are being extensively studied as highly promising platforms for the delivery of molecules such as insulin or nucleic acids. MNs also show promising possibilities in the field of vaccine delivery. The application of MNs in ophthalmology is still in its early stages, with much promise but also numerous unresolved issues [13]. Most eye disorders are treated with drops or ointments, which have the problems of needing to be administered frequently, having low bioavailability, and being unable to traverse the numerous eye barriers. Because of this, unconventional delivery methods are required. Barriers associated with ocular drug delivery hamper the delivery of the correct amount of drug reaching the desired point of contact.

Microneedles (MNs) are revolutionary alternative delivery technologies that show potential in the treatment of eye disorders [14]. Owing to developments in pharmaceutical science, MNs can administer drugs precisely where they're needed, in the eye, with minimal risk to the patient. This chapter aims to shed light on the potential therapeutic benefits that MNs can bring to the treatment of a wide range of ocular diseases. The potential applications of MNs in ocular diseases are particularly focused on solid-coated MNs, hollow MNs, and dissolving polymeric MNs [15]. Implication of therapeutic potential of various types of microneedles is shown in Fig. 1.

2 Types of microneedles

2.1 Types of microneedles and properties

Microneedles applied for drug and vaccine delivery, as well as in diagnostics, can be classified according to several different criteria (Fig. 1). The most common microneedle typifications are based on the geometry, the material applied to obtain the systems, the method of fabrication, the drug loading technique, and the mode of drug delivery [16]. Concerning the materials used to manufacture the microneedle arrays, the range of available substances is wide. The first material used for this purpose was silicon. It is important to note that it offers versatile properties and the ability to form different microneedle geometries, which can be considered as an advantage. However, the manufacturing process can be complicated, and the material is relatively expensive. Also, silicon is a brittle, noncompatible, and nonbiodegradable

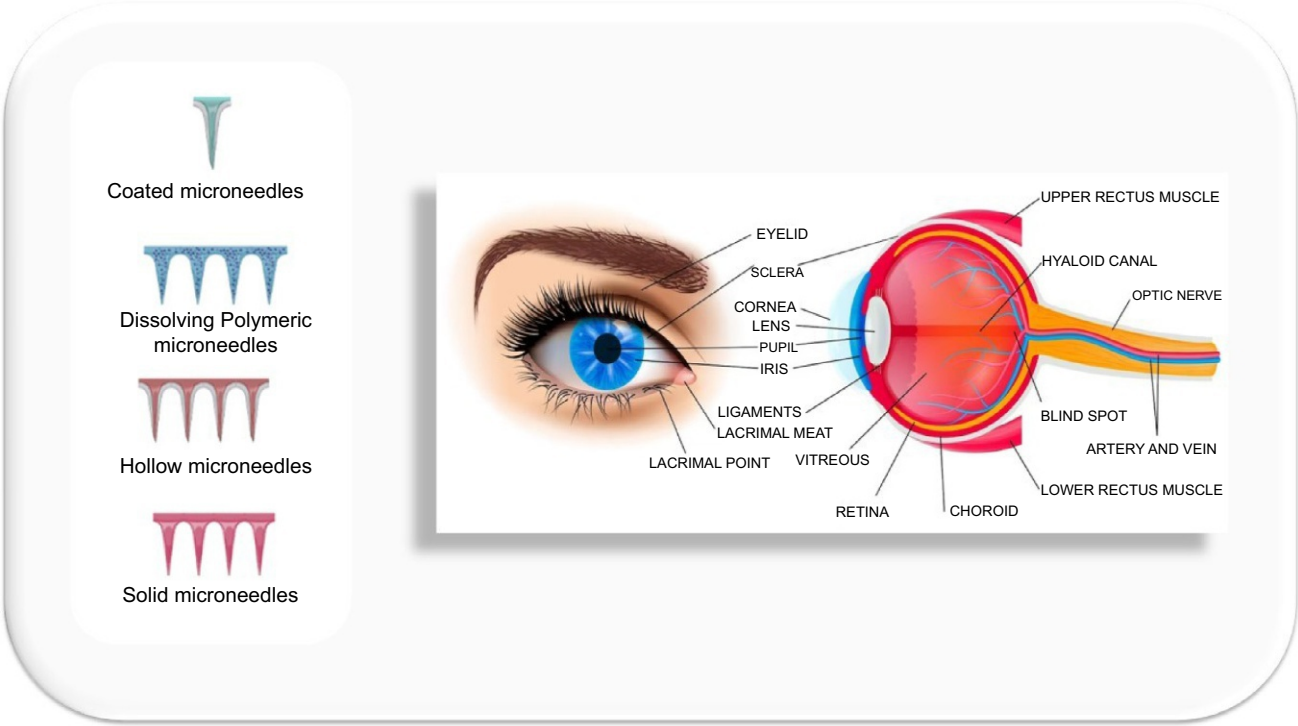

Fig. 1 Schematic representation of implication of therapeutic potential of various types of microneedles.

material, which may cause skin irritation if the needles break and are deposited in the tissue [17]. Other nondegradable materials utilized to fabricate microneedles include metals such as stainless steel and titanium, ceramics, such as aluminum oxide, or synthetic polymers comprising polyvinylpyrrolidone (PVP), polyvinyl alcohol (PVA), and polymethacrylates. Among the biodegradable materials, carbohydrates, including maltose, trehalose, and sucrose are frequently mentioned. Moreover, biodegradable polymers such as poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and poly(lactic-co-glycolic)acid (PLGA) are widely investigated as microneedle materials. Among them, there are hydrogel-forming agents swelling upon contact with interstitial fluid in the skin during microneedle application [18–21]. These polymers include poly(ethylene glycol) diacrylate (PEGDA) and poly(acrylic-co-maleic) acid (PAMA) [22]. It is also important to notice that there are numerous studies describing the use of composite materials containing combinations of various substances, both organic and inorganic. For example, studies involving PLA and carbon nanotubes, calcium sulfate and gelatin, gelatin and hydroxyapatite and PLGA microparticles combined with PLA are available in the scientific literature [23]. Various types of microneedles are shown in Fig. 2.

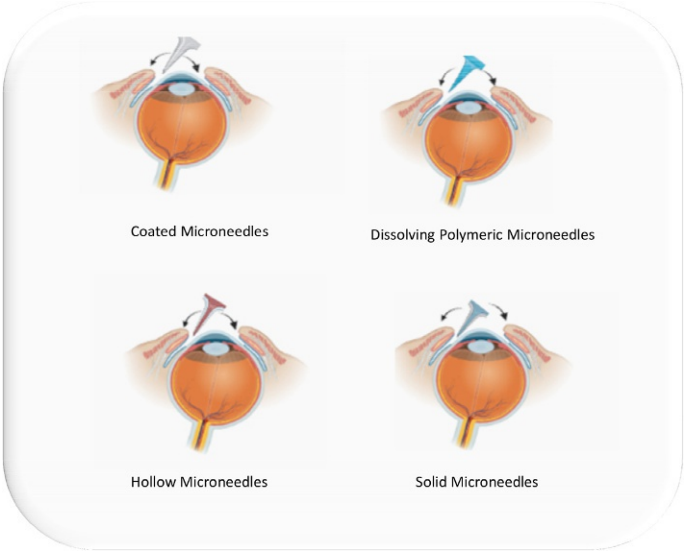

Fig. 2 Depiction of mechanism of various types of microneedles used in ocular drug delivery.

2.1.1 Solid-coated microneedles

Solid microneedles are generally used in vaccine delivery, have robust response, facile produce along with better mechanical characteristics. Based on the mechanism of action, these types of carriers perforate inside the stratum corneum to maximize drug action inside the dermis layer and enhance the bioavailability and half-life of drug inside the skin. The additional feature of coating of microneedles with the drug solution makes it denser with a minimalistic delivery of drug at a controlled pace and is useful in gene delivery. Interestingly, solid microneedles made up of silicon have been designed and later gold-coated for improving mechanical properties for efficient skin delivery. The constructed needles were of height of 250 μm and a base width of 52.8 μm, the aspect ratio of 4.73, and tip angle and diameter of 24.5 degrees and 45 μm for sustained drug action [24]. In another study, 12 groups of uncoated microneedles (u-MNs) were envisaged from the amalgam of three various aspect ratios and four different synthetic polymers (polyethylene (PE), polypropylene (PP), nylon, and polylactic acid (PLA)). After coating the u-MNs with polyvinyl alcohol formulation to make coated MNs (c-MNs), the aspect ratio was reduced from 2.2, 2.5, and 3.0 with u-MNs to 1.3, 1.4, and 1.6 with c-MNs, respectively. All PLA MNs had a puncture performance of more than 95% based on animal studies [25].

Hollow microneedles

Hollow microneedles are notably promising to extract skin interstitial fluid (ISF) for detection, used for sensing biomarkers as well as facilitating diagnosis. In a recent research study, a facile technique for hollow microneedles on the foundation of the refraction index variations of polyethylene glycol diacrylate (PEGDA) was demonstrated in the process of photopolymerization. The designed system showed excellent mechanical characteristics to inject the dermal layer by capillary force. The system showed positive outcome in diabetes management [26].

Dissolving polymeric microneedles

Dissolving polymeric microneedle is one of the most potential microneedle patches owing to rapid higher-molecular-weight drug release, patient compliance and is considered a good option among others for effective action. The dissolvable microneedle tip can be filled in a timely manner, and upon injecting this into the skin, the drug load releases and diffuses simply by dissolution of the needle tip. Water-soluble constructs are considered suitable for synthesis and nevertheless require expert hand. In this direction, Sadeqi

and group presented a polymer-based porous microneedle patch system bearing Lidocaine and Ibuprofen for easy skin delivery. In the work, larger area ($6 \times 20\,cm^2$), flexible as well as stretchable microneedle patches capable of delivery of drug on any body part were also observed [27].

Hydrogel-forming microneedles

These types of microneedle patches are appropriate for larger doses and comprise hydrogel-containing drug that encounters fluids inside the dermal layer, gets hydrated, however, does not get dissolved; and these patches release drug through diffusion at a slower rate. Recently, a smart microneedle patch complex has been investigated for minimally invasive glucose monitoring. This pH-sensitive as well as glucose-sensitive hydrogel incorporated a photocross-linkable gelatin methacrylate (GelMA) together with a pH-responsive nanogel (nano(CMC-pHEA)) and glucose oxidase (GOx). The composite hydrogel observed better action along with increased responsiveness toward glucose levels in desired range [28]. In a different study, a poly(vinyl alcohol) (PVA)-based mixture was used to construct hydrogel-forming microneedles bearing good surface stability and outstanding needle development with high performance [29]. In another concomitant study, a glucose-sensitive insulin-liberating hydrogel for microneedle dressing has been constructed and analyzed in diabetic wound healing process. This system incorporated gelatin methacrylate, 4-(2-acrylamidoethylcarbamoyl)-3-fluorophenylboronic acid (AFPBA) along with G-insulin resulting in better mechanical characteristics and showed promising outcome in other adverse dermal injuries [30].

2.2 Microneedle manufacturing methods

The intended purpose of the microneedle system and the material from which it is to be formed should be considered while selecting a manufacturing procedure. Furthermore, the approach must be tailored to the qualities of the medicine. Silicon, ceramics, and metals such as stainless steel and titanium are the most commonly utilized materials in the manufacture of microneedles. Biodegradable polymers such as poly(lactic acid) and poly(glycolic acid) are also used, as are nonbiodegradable polymers such as photolithographic epoxy resins [30]. Microneedle production methods include lithographic or laser processes, casting, and 3D printing, to name a few. Laser cutting can be used to obtain microneedles out of metals or polymers. The main portion of the procedure involves laser-cutting microneedles from a plate and then bending them. Electropolishing is a method

for aligning the tips. Laser ablation is a comparable procedure. In this situation, the substrate absorbs the laser beam and heats up, causing it to evaporate or sublime, resulting in etched 3D patterns [31]. The thermoplastic material is heated to its softening point before being extruded through a nozzle and deposited layer by layer to the build plate, where it instantly hardens. A prepared polymer or polymer mixture is polymerized in the microstereolithographic process under the influence of a high-energy light source (e.g., UV radiation) [31]. Digital light processing (DLP) is also a technology based on photopolymerization of photosensitive polymers, but in this case, each layer of the polymer is projected as whole. A more complicated method is two-photon polymerization (TPP), which uses a near-infrared beam instead of UV radiation. TPP initiates the polymerization of the resin by multiphoton absorption [32].

The droplet-born air blowing method (DAB) is an alternative to procedures that use UV or heat. It is appropriate for inactivatable medicinal compounds. Polymer droplets are inserted between two sheets in this process. The droplets extend as the sheets are dragged apart, and the resulting needle-shaped matrices are dried by the flowing air [32]. Photolithography is another well-known technique. A silicon wafer is covered with a photosensitive or photoresistant polymer in this approach. After that, the plate is exposed to UV rays. Depending on the coverage of the photosensitive or photoresistant layer, a pattern is formed. After that, the wafer is etched. There is a distinction between wet and dry etching. Wet etching uses a potassium hydroxide solution, whereas dry etching uses the physical methods of ion milling and sputtering, as well as the chemical method of high-pressure plasma [33]. Molds for the micromolding technology can also be prepared using lithographic techniques. Micromolding, also known as solvent casting, is quite popular due to its low cost and ease of use. This approach makes use of a silicone mold. A polymer solution or mixture is poured into the prepared mold. The mold is then baked in the oven after air spaces are eliminated with a centrifuge or vacuum. The final microneedles are taken from the matrix after cooling. This approach is used to obtain microneedles for ocular applications [34].

The drug-loading methods used are determined by the type and construction of the created microneedles, as well as the material used. The drug's qualities must be carefully considered, as some active components may degrade at higher temperatures or after irradiation. In the case of porous ceramic materials used to obtain solid microneedles, the pores in the carrier material can be filled with the active ingredient in liquid or solid form. In the

first situation, when the medicine is applied, it diffuses from the solution into the microneedle holes. In the other situation, the drug solution is placed onto the microneedles, and the formulation is then dried, allowing the drug to precipitate inside the pores. When applied, the medication dissolves in a physiological fluid and can then enter deeper tissues [35]. The medicine may be dissolved or suspended in the polymer or monomer solution in processes utilizing polymers used to produce solid microneedles. More complicated techniques utilizing both drug-loaded and drug-free fluids may be used in the manufacture of more complex systems, with the active ingredient integrated in a specific compartment of a microneedle. Hollow microneedles with an empty canal inside are often filled with active component solution passively or through pressure-driven methods [36]. The drug solution can be loaded into the canals within the system or into an external compartment in passive diffusion systems. A reservoir with various pumping mechanisms is connected to the microneedle systems in pressure-driven systems. Typically, manually operated syringes, as well as micropumps or actuator-equipped devices designed for self-injection, are recorded. The medication is deposited as a thin layer on the microneedle's exterior surface in coated microneedles. Various coating procedures have been reported and summarized in the literature [36]. The most basic approach is dip coating, in which the microneedle array is dipped in the coating liquid and then allowed to dry. The drying process can be sped up by using gas-jet drying. Another method is spray coating, which is similar to the coating of oral solid dosage forms in terms of technology. Electrohydrodynamic atomization (EHDA), which involves depositing electrically charged droplets on the microneedle surface, has been mentioned. As with piezoelectric inkjet printing, droplets are released from the nozzle as a result of an electrical field being applied to it.

3 Advantages and disadvantages of microneedle systems for ophthalmic drug delivery

Microneedle arrays as prospective drug delivery systems have several advantages, including ease of administration without expert assistance, the capacity to bypass exterior human body barriers that reduce the effectiveness of topical formulations, and low invasiveness. Poor bioavailability is one of the most significant difficulties in the administration of ocular drugs [37]. Specific physiological circumstances in this body location are responsible for rapid drug clearance from the surface of the eyeball and relatively brief formulation residence periods after delivery. Because of its poor accessibility

and inherent ocular obstacles, reaching the posterior eye segment is much more difficult [38]. According to existing scientific research, microneedles may provide certain therapeutic benefits over standard formulations, as well as improved patient acceptance when compared with traditional intraocular injections. It should be noted, however, that dealing with this sensitive organ is normally connected with potential discomfort, especially in the case of comparatively mild formulations such as eye drops, gels, and contact lenses. It is also worth noting that, despite the fact that the described microneedle systems are minimally invasive, there is a danger of infection and inflammation at the administration site, which may cause further discomfort and anguish. One of the most promising avenues appears to be the development of patches that are easily detachable or dissolvable. This is because leaving the patch on the surface of the eye for an extended period of time is bound to induce discomfort in patients. It is also incredibly intriguing to be able to make such patches utilizing 3D printing techniques, which not only allow the recurrent injection of suitable doses of APIs but also allow the amount to be customized to the needs of a certain condition and patient. Clinical trials involving human volunteers are critical for determining the actual relevance of the suggested systems, as Dugam and colleagues [39] have already stated. To the best of our knowledge, all completed clinical studies involving microneedle-based formulations have used single microneedle devices rather than microneedle arrays. However, to the best of our knowledge, all completed clinical studies related to microneedle-based formulations do not involve microneedle arrays but single microneedle devices [38]. It is evident that additional research is required to specify formulation development directions and identify the most significant constraints of these systems. The currently accessible reports primarily address formulation studies and the outcomes of in vivo experiments using animal models. It should be noted that the results are often encouraging and demonstrate the potential for scientific and therapeutic development of microneedle arrays.

For ocular medication delivery, several techniques have been used, including systemic, topical, periocular (or transscleral), and intravitreal (IVT) routes. Because of the presence of several ocular barriers, the use of topical techniques, such as eye drops, and systemic administration, such as oral tablets, has resulted in inadequate or subtherapeutic drug concentrations. As a result, excessively high drug concentrations must be administered through these methods, resulting in drug-related toxicity and decreased therapeutic effectiveness [29,30]. To overcome the ocular barrier and optimize drug localization in close proximity to the target tissues, injections are

administered through a variety of routes, including directly into the eye (intravitreal, IVT), around the external surface of the eye (periocular or transscleral route), and within the ocular tissues themselves (intracorneal and intrascleral). The use of ordinary hypodermic needles facilitates the administration of these injections. While the periocular approach is considered less intrusive than the IVT method, it is important to note that transient drug diffusion across the scleral layer remains limited. Several factors influence drug penetration through the scleral membrane, including drug solubility, molecular weight/radius, charge, and polarity [38]. Nonetheless, the method's intraocular bioavailability has been significantly reduced due to delays in diffusion through the sclera, systemic clearance, and premature drug loss before reaching the desired target tissues such as the retina [39]. To alleviate the limitations of periocular injections, a commonly used technique involves either IVT for posterior segment illnesses or intracorneal injections for anterior region ailments. However, injecting typical hypodermic needles into the eye, whether targeting the anterior or posterior portions, causes severe discomfort and agony and necessitates a unique skill set. It is important to note that the repetitive and protracted usage of hypodermic needle-based injections can potentially increase the risk of severe ocular problems and decrease patient adherence. As a result, there is a major demand for less intrusive techniques that not only increase patient adherence but also allow for accurate and targeted medication administration to ocular tissues [40]. In this context, microneedles (MNs) have emerged as a unique technology, altering the landscape of transdermal medication and vaccine administration. MNs, when applied to ocular medication administration, provide a minimally invasive yet effective approach. MNs excel in delivering drug-loaded formulations with greater precision and accuracy than standard hypodermic needles because they accurately target unique ocular regions. Table 1 lists several ocular diseases, their symptoms, and treatments.

3.1 Glaucoma

Glaucoma, a multifactorial disease, stands as the primary cause of irreversible blindness, impacting an estimated global population of 70 million. This condition arises from the dysfunction of one or more peripheral nerves in the eye and is distinguished by both genetic and biological risk elements. The hallmark features encompass the degeneration of the optic nerve, leading to the characteristic appearance known as cupping, along with the demise of retinal ganglionic cells, culminating in eventual visual field deterioration

Table 1 Summary of various ocular diseases, their symptoms, and treatments.

Disease	Symptoms	Treatment
Age-related macular degeneration	Breakdown of photoreceptors, retinal pigment epithelium (RPE) and choriocapillaries	Formulation containing antioxidants, zinc and vitamin supplements
Diabetic macular edema	Accumulation of excess fluid in the extracellular space within the retina in the macular area, typically in the inner nuclear, outer plexiform, Henle's fiber layer, and subretinal space	Focal or grid lasers and steroids
Proliferative vitreoretinopathy	Simple scar formation and proliferation of cells in vitreous and retina	Surgery and adjunctive treatment after surgery, to avoid relapses
Uveitis	Inflammation occurs in the middle layer of eye (uvea)	Corticosteroids and immunosuppressive agents
Glaucoma	Obstruction to the outflow of aqueous humor from the anterior segment	Prostaglandin analogues, beta-adrenergic receptor antagonists, alpha-2 adrenergic agonists, parasympathomimetics, carbonic anhydrase inhibitors
Conjunctivitis	Infection of conjunctiva, redness, irritation, grittiness and watering of the eyes	Antihistamine and nonsteroid antiinflammatory agents and bacterial conjunctivitis is treated using antibiotics and corticosteroids

[29,38]. Glaucoma is primarily characterized by elevated intraocular pressure (IOP) as its major contributing factor. Intraocular pressure (IOP) is essentially the equilibrium between the production of aqueous humor by the ciliary network and its drainage through the internal outflow system [30,39]. The stress exerted on the lamina cribrosa by intraocular pressure can lead to significant strain on this mesh-like structure, which houses nerve fibers and eventually gives rise to the optic nerve. This stress-induced pressure on the lamina can bring about disarrangement and distortion, culminating in the detrimental effect of retinal ganglionic cell damage [41].

Glaucoma's secondary causal factors encompass oxidative stress, compromised immunity, and more. Within the ocular system, two distinct internal outflow pathways exist: the trabecular meshwork system and the uveal meshwork system. The trabecular meshwork system comprises connective tissues and functions as a bridge spanning the scleral sulcus – the groove situated between the sclera and the cornea. The primary pathway responsible for the outflow of aqueous humor is the trabecular pathway, which operates through the structure known as Schlemm's canal. This conventional pathway facilitates the drainage of aqueous humor into both the episcleral and aqueous veins [42]. In contrast, the unconventional pathway, also referred to as the uveal meshwork system, involves the passage of aqueous humor through the sclera, subsequently draining into the episcleral and aqueous veins as well [42,43]. The condition of glaucoma presents in two primary forms: primary open-angle glaucoma and closed-angle glaucoma. Within primary open-angle glaucoma, certain conditions emerge, such as heightened resistance to aqueous humor outflow and obstruction within the trabecular meshwork pathway [44]. Conversely, closed-angle glaucoma results from an obstruction in aqueous flow due to the iris, leading to an escalation in intraocular pressure and consequential damage to the optic nerve [38]. The treatment for glaucoma encompasses the utilization of topical agents. Various classes of drugs are used in addressing glaucoma, such as alpha adrenergic agonists, beta blockers, cholinergics, carbonic anhydrase inhibitors, and prostaglandin analogs. In instances of heightened severity, surgical interventions become necessary. Trabeculectomy is conducted for open-angle glaucoma, while iridotomy is the procedure of choice for closed-angle glaucoma [45]. In the realm of glaucoma treatment, emerging therapies have begun to overshadow conventional approaches. Notably, microneedles (MNs) have gained prominence, particularly when targeted at the supraciliary region to facilitate efficient drug delivery. Despite this, ongoing research continues to explore various medications and administration routes for glaucoma. A study by Jiang et al. presented a novel approach used coated stainless steel MNs to administer drugs to the anterior segment of the eye, addressing glaucoma. By utilizing an intrascleral route for pilocarpine delivery, they achieved a remarkable 45-fold increase in drug absorption efficiency [46]. Intriguingly, this approach exhibited a striking 60-fold enhancement in fluorescein delivery compared with topical methods. The MNs used in this study measured between 500 and 750 μm in length. Furthermore, the research delved into the utilization of hollow MNs for intrascleral delivery of sulforhodamine. Fabricated from borosilicate micropipette tubes, these hollow MNs enabled

precise drug administration at concentrations ranging from 10 to 35 μL per individual MN within the array [40,41]. This innovative exploration signifies a shift toward more effective and targeted glaucoma treatments, illustrating the potential of MNs in advancing ocular drug delivery.

3.2 Macular degeneration

AMD is a condition that occurs predominantly in the elderly, characterized by vision loss resulting from macular degradation, the central portion of the retina responsible for sharp vision [43]. This degradation affects not only the macula but also leads to the deterioration of the retinal pigment epithelium membrane, the outer layer of the retina [44]. Consequently, the photoreceptor cells, including cones and rods, experience loss and subsequent cell death, ultimately resulting in profound symptoms, notably complete vision loss [47]. It is an acquired condition affecting millions worldwide and has associated risk factors including family history, age, sun exposure, ocular factors, oxidative stress, and smoking [48]. Its classification comprises dry AMD involving drusen formation near retinal pigment epithelium and wet AMD characterized by weak, leaky neovascularization [49]. This condition's treatment involves the use of anti-VEGF (vascular endothelial growth factor) agents. VEGF, a glycoprotein, plays a crucial role in the development and maturation of blood vessels, while also regulating vascular permeability by binding to endothelial cell receptors [50]. The excessive expression of VEGF in AMD patients leads to the emergence of abnormal blood vessels that are prone to leakage, subsequently resulting in hemorrhage during disease progression [51]. To counter the formation of these problematic blood vessels, intravitreal injections of anti-VEGF therapies such as Pegaptanib, Ranibizumab, and Bevacizumab are administered [52–59]. Photodynamic treatment (PDT) involves the utilization of a photosensitizing drug, verteporfin (Visudyne), administered intravenously for 10 min. This is accompanied by the application of a cold laser with a wavelength of 689 nm to the eye for 15 min, aimed at exciting and activating the drug [60,61]. The activated drug induces damage to either the endothelial cells or the abnormal blood vessels within CNV. Recent literature highlights that PDT, in certain instances, can lead to an upregulation of VEGF expression, potentially necessitating the concurrent use of anti-VEGF therapy alongside PDT [61]. Laser photocoagulation, using argon and krypton lasers, presents another treatment approach. These lasers generate rays of sufficient energy to induce thermal reactions. These reactions lead to the coagulation and

blockage of abnormal blood vessels [6]. Another innovative treatment involves the use of tower microneedles (MNs) through intravitreal injection to administer antivascular endothelial growth factor antibody for managing the exudative form of AMD or neovascularization This study compared the effects of intravitreal injections performed with both hypodermic needles and tower needles. Hypodermic needles, with gauges ranging from 27 to 30, are commonly used in neovascular AMD treatment [62]. However, their larger outer diameter increases the risk of surgical complications such as hemorrhage, retinal detachment, and improper drug distribution [63,64]. To address the challenges tied to hypodermic needles and their associated complications, a novel needle variant known as the "tower needle" has been innovated. Tower microneedles (MNs) possess a slender outer diameter yet adequate length for scleral penetration. In a conducted experiment, these MNs facilitated neovascularization induction in a mouse model of age-related macular degeneration (AMD) through laser intervention. Subsequently, the same tower MNs were used to administer anti-VEGF antibody therapy. The evaluation of bleb dimensions in both scenarios was established by intravitreal injection of sodium fluorescein, followed by size measurement [65]. Upon comparison, it was determined that hypodermic microneedles (MNs) induce bleb formation, signifying the creation of a minor aperture, referred to as a scleral hole, during needle insertion. This opening establishes a conduit allowing vitreous humor to flow from the vitreous chamber to the subconjunctival space, resulting in fluid accumulation known as a bleb [65]. In contrast, the use of tower MNs yields minimal or no bleb formation due to their reduced outer diameter, accompanied by the absence of surgical complications typically associated with these microneedles [66].

3.3 Uveitis

Uveitis, a comprehensive term encompassing inflammatory and infectious ocular conditions, is a significant contributor to visual impairment, ultimately leading to blindness. The uvea, comprising the iris, choroid, ciliary body, and associated structures including the retina, sclera, and optic nerve, constitutes the inner ocular lining [67,68]. Uveitis, classified into anterior, intermediate, posterior, and panuveitis, designates inflammation occurring at distinct ocular sites. Specifically, anterior uveitis targets the anterior chamber, intermediate uveitis affects the vitreous, posterior uveitis involves the retina or choroid, and panuveitis encompasses all three sites—anterior

chamber, vitreous, and retina or choroid [69–72]. Infectious uveitis stems from various sources, including toxoplasmosis, leptospirosis, onchocerciasis, and cysticercosis. Conversely, noninfectious uveitis is linked to conditions such as heterochromic iridocyclitis and chorioretinopathy [73,74]. The treatment of the disease necessitates various medications, encompassing immunosuppressive agents such as corticosteroids, cytotoxic drugs such as chlorambucil and cyclophosphamide, biological agents such as etanercept, infliximab, and adalimumab targeting TNFα, as well as noncorticosteroid options such as Bevacizumab, an anti-VEGF therapy [75]. In addressing the complexities of administering medication to the posterior segment of the eye, Gilger et al. innovatively harnessed the potential of microneedles (MNs) for the treatment of acute posterior uveitis. Their approach involved utilizing hollow MN to deliver triamcinolone acetonide (TA) within the suprachoroidal space (SCS), which is the anatomical region between the choroid and sclera adjacent to the posterior eye segment. This inventive method was assessed using a porcine model to investigate TA's impact on acute posterior uveitis. Notably, the study's outcomes revealed that a 2 mg dose of TA effectively mitigated posterior inflammation for a duration of up to 3 days. Remarkably, this approach exhibited no indications of retinal toxicity or increased IOP following SCS injection [76,77].

3.4 Retinal vascular occlusion

Retinal vascular occlusion, a retinal condition resulting in vision loss, occurs due to inadequate blood supply, oxygen, nutrient delivery, and waste removal necessary for optimal retinal function. The retinal vascular system consists of multiple arteries and veins. Blockages or clot formation in these vessels leads to a condition termed occlusion [78–82]. In the context of treating retinal vein occlusion, Kadonosono et al. introduced a novel approach using microneedles (MNs) for retinal endovascular cannulation. Traditionally, glass cannulas were utilized to address this condition, but due to concerns such as fragility and associated complications, the researchers devised a fresh methodology involving MNs to effectively dilate the occluded retinal vein [83,84]. In this investigation, a stainless steel microneedle (MN) with a diameter of 50 μm and an inner diameter of 20 μm was fabricated. The MN was affixed to a syringe containing a saline solution of 10 μL, designed to puncture the occluded central retinal vein and extract thrombotic material within. The study encompassed 12 patients afflicted with this condition. The outcomes revealed minimal to no complications, indicating the efficacy of

the surgical MN intervention in enhancing visual acuity and addressing the ailment. Another recent study also reported the feasibility of endovascular cannulation using microneedles (MNs) for the treatment of retinal artery occlusion [85]. The study enrolled 13 patients with CRAO, employing tPA surgery through a 47-gauge stainless steel MN attached to a syringe for effective embolism treatment within the artery. The procedure involved vitrectomy followed by successful tPA injection using the syringe-attached MN. Notably, 85% of patients exhibited improved visual acuity postcannulation, with only one case experiencing vitreous hemorrhage as a complication. The study's outcome underscores the success of endovascular cannulation utilizing MN for CRAO treatment, particularly when promptly performed upon symptom onset [86,87].

3.5 Retinitis pigmentation

Retinitis pigmentosa, an inherited ocular disease, encompasses various heterogeneous conditions stemming from damage to both rod and cone cells. Primarily impacting rod cells, it later extends its influence to cone cells, resulting in the ailment's alternative name, rodcone dystrophy. A hallmark of RP is the alteration in photoreceptor cell pigmentation, evident in the early stages as bone-spicule pigmentation. Initial symptoms include night blindness due to rod cell dysfunction, followed by peripheral vision impairment as the disease progresses. The macula and fovea impairment contributes to cone cell dysfunctioning [88–91]. Retinitis pigmentosa is classified into syndromic and nonsyndromic forms. The nonsyndromic form arises from mutations in various genes, while the syndromic form involves both ocular and systemic manifestations. The nonsyndromic category comprises autosomal dominant RP, autosomal recessive RP, and X-linked RP, categorized based on gene types [92–94]. The focus of this study was to address the treatment of retinitis pigmentosa caused by a mutated autosomal dominant RHO (rhodopsin) gene. An AAV-based vector was used to deliver RNA interference (RNAi) aimed at suppressing the mutant gene while simultaneously introducing a replacement gene. To simulate the human RHO autosomal dominant gene, a transgenic mouse model was utilized [95,96]. The efficient delivery of the vector was achieved by injecting it into the subretinal space using a 34-gauge MN attached to a syringe [97]. In a novel study, the replacement of a mutated gene in a mouse model effectively suppressed and enhanced the evaluation process of electroretinography (ERG)—a technique measuring the retinal, inner retinal, and

photoreceptor cell electrical signals. The findings indicate the method's efficacy in mitigating disease progression, particularly retinal degeneration, and its potential applicability to various prevalent genetic disorders [97–101].

4 Conclusion and future prospectives

Delivering medication to the eye presents challenges due to ocular barriers and limited effectiveness of conventional methods. Insufficient drug penetration in these forms necessitates intraocular injections through conventional hypodermic needles, involving different routes such as intracorneal, intravitreal, periocular, and supraciliary delivery. However, these needles are associated with drawbacks such as frequent administration, invasiveness, and low bioavailability. Micron-sized MNs offer a solution, overcoming these issues and emerging as an effective technology for ocular drug delivery. These MNs not only halt disease progression but also curtail retinal degeneration. Solid-coated, hollow, and dissolving polymeric MNs are utilized for ocular therapy, with promising applications in glaucoma, macular degeneration, uveitis, retinal occlusion, and more. Studies demonstrate MNs' potential in slowing eye disease progression, positioning them as a promising therapy, even for genetically linked cases.

The reviewed studies highlight the undeniable advantages of microneedles as ocular drug delivery systems, alongside significant drawbacks. Current trends primarily emphasize rapidly dissolving systems achieved through hydrophilic polymers. These formulations aim for brief eye surface residence to minimize discomfort and potential side effects such as irritation, tissue damage, infection, and inflammation. However, the available data on potential risks associated with ophthalmic microneedles remain insufficient, necessitating further exploration. Given the need for in-depth safety analysis, there are no ongoing endeavors to introduce microneedle-based ophthalmic formulations into the pharmaceutical market, except for those utilizing single microneedles instead of arrays. Nevertheless, valuable insights can be derived from studies on single microneedle injections, wherein factors such as injected volume influence perceived pain or acceptability [97]. Careful consideration and optimization of these factors are imperative. Furthermore, the challenge of maintaining sterility poses a significant concern for ophthalmic drug delivery systems. Achieving stability for both the drug and applied polymers necessitates the selection and optimization of appropriate sterilization methods. Regrettably, this issue has not been comprehensively addressed, as evidenced by the limited scientific literature on the subject. To advance in this field, randomized clinical studies using microneedle arrays are also warranted.

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CHAPTER 13

Microneedles deliver drugs to ophthalmic tissue

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1 Introduction

The eye is one of the most sensitive, exclusive organs in the human body, and it accounts for visual senses [1]. Ocular disorders and diseases represent a group of diseases that influences the function and health of the eye. These attack millions of individuals around the world, and it is estimated that by 2050, 895 million people will have distance vision impairment, and 61 million of them will be blind [2]. Cataracts, glaucoma, age-related macular and diabetic retinopathy, infection, uveitis, and dry eye are the most common eye diseases; these are caused by pathogenic infections, complications of systemic disease, genetics, the environment, and old age [3]. Most of these diseases can be diagnosed and partially treated, but irreversible changes can occur and lead to blindness if treatment is inappropriate or late [4].

The physiological barriers that protect the eyes are the cornea and anterior segment barriers, the sclera and Bruch's-choroid complex, and the blood-retinal barrier; however, such barriers also remain a key obstacle in terms of ocular drug delivery due to the limitations of active agents gaining entry into the eye [5]. Normally, drug therapy for anterior ocular disorders is done by topical drug administration with some pharmaceutical dosage form in a clinical setting and by individuals through gels, eye drops, ointments, and suspension liquids. Although such drug delivery systems represent more than 95% of marketed products, they have <5% of bioavailability due to insufficient corneal permeation and short residence time [6]. In case of posterior segment disorders, injection administration is a useful means with different techniques such as intravitreal injection, periocular injection, and suprachoroidal injection. Poor patient tolerance, relevance of surgical

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intervention, and increased side effects hinder the use of injections for ocular drug delivery [7]. To increase bioavailability as well as decrease adverse effects, several methods have been developed, including a microneedle (MN), iontophoresis, electroporation, sonophoresis, inserts, and contact lens with active ingredients [5,8,9]. While some methods are limited in their technique by the usage, the MN for ocular drug delivery has emerged as a feasible application because of the large amount of research on MNs. The diversity of morphology, fabrication methods, and materials as well as the development of microsized facilities and mass production have helped in applying MN to treat eye diseases. This chapter summarizes MN research in ocular therapy in detail, including the MN type and administration site. In addition to the eye structure and drugs for ocular disease, we also give a comprehensive overview of MN for ocular drug delivery.

2 Eye structure, disease and treatment

The eyes consists of two large parts, the anterior and posterior segments. The anterior area is composed of the tear film, cornea, pupil, lens, and ciliary body, which account for the outer look of the eye. The posterior segment is the inside part of the eye with the sclera, choroid, retina, vitreous humor, optic nerve, and blood vessel [10] (Fig. 1). Most anterior segmental diseases occur at the cornea, which contains the three cellular layers in the epithelium, stroma, and endothelium as well as two interface layers in the Bowman's layer and Descemet's membrane. Also, there are no blood vessels in the cornea. Additionally, the thickness of the cornea is about 50 μm, and this plays an important protective role. The pupil, iris, and lens are responsible for adjusting light to the retina and the visual function of the outer tissues. In front of the cornea, the tear film layer is the constant flow of tear fluid across the outer surface of the eye; it is replaced every 2–20 min and is approximately 3–10 μL in volume [11]. The tear film is structured with three layers: the lipid layer, the aqueous layer, and the mucin layer (Fig. 1). The conjunctiva is a vascularized mucus membrane that includes two layers: an outer epithelium that works as the main penetration barrier and its underlying stroma. Topical dosage forms such as eye drops, ointments, and gels are the most popular dosage form to apply to the anterior segment to treat ocular disease such as glaucoma, infection, fungal keratitis, and conjunctivitis; these are applied by medical staff or by the patient [12].

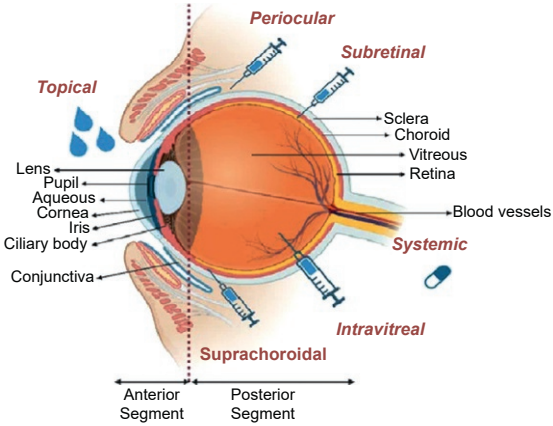

Fig. 1 Anatomy of the eye: Eye with two main areas are anterior segment and posterior segment and the main route of drug administration to the eye as topical, periocular injection, subretinal injection, systemic injection, intravitreal injection and suprachoroidal injection. Preprinted by permission of [10].

In terms of the posterior segment, the sclera is composed of randomly scattered collagen fibers and has a thickness of 0.5–1 mm. This is the main barrier preventing drug permeation to the posterior, and the choroid accounts for drug elimination. Next to the choroid is the retina, which is a thin transparent tissue that consists of the outer monolayer of polarized cells and the inner neural retina with nine layers containing extracellular matrix protein, posing the most significant barrier to drug penetration [13]. The vitreous chamber is a semisolid structure that helps maintain the shape of the retina. To treat diseases associated with the posterior segment, the drugs need to reach the macular or peripheral retina; injection administration has been mostly used. Each component of the eye has different characteristics for active ingredients to go into and eliminate (Table 1). The degree of drug absorption depends on the time they remain at the application site and their properties to help overcome the natural barriers of the eye. Such special aspects make ocular drug delivery challenging to achieve.

Due to sophisticated physiology and anatomy, ocular diseases and treatment drugs also one of the most consideration for ocular health care around the world, with cataracts accounting for 40%–60% of blindness in the world;

Table 1 The components of the eye and their characteristics for drug delivery.

Components	Drug delivery properties	Refs.
Tear film	– Mucin: prevents exogenous substances permeating to the deeper tissues	[14]
	– Irrigation with fluid removes the drugs	[11]
Cornea	– Permeable substances <1 kDa, molecular radius <5.5 Å	[15]
	– Cationic compounds permeate more easily across the cornea when it is charged above the isoelectric point	[16]
	– Epithelium: passage of small and lipophilic drugs	[17]
	– Stroma: passage of hydrophilic drugs	[18]
	– Endothelium: prevents hydrophilic drugs and macromolecules from passage into the aqueous humor	
Conjunctiva	– Drug permeates more than across the cornea because of larger surface	[19]
	– Drug loss into systemic circulation through the conjunctival blood capillaries and lymphatics	[5]
Sclera	– Well permeable to substances <70 kDa	[14]
	– Drug elimination via nasolacrimal drainage pathways, tear protein binding, drug metabolism	[20]
Eyeball and eyelids	– Constant movement to remove foreign agents	[21]
Retina	– Larger molecules have difficulty penetrating	
	– Retinal pigment epithelium reduces (a single layer of cells tightly joined and form barrier between retina and underlying choroid) drug diffusion because of increasing radius is permeability declining exponentially	[5]
	– Only small lipophilic molecules could transfer between choroid and retina	

moreover, vision impairment can be caused by aging, diabetes, glaucoma, and fungal infections. The characteristics, cause, and treatment of such ocular disorders are summarized in Table 2.

3 MN strategy for opthalmic drug delivery

3.1 Single MN (hollow, pen-based MN)

Single MNs are made of one needle; they are about 25–2000 μm in length with the hollow-type, pen-based MN and the solid type MN. To deliver active agents inside the eye, the injection has been done by conventional

Table 2 Common ocular disease and agents for treatment.

Ocular disease	Characteristics and causes	Treatments	Refs.
Age-related macular degeneration	It is multifactorially degenerative related to the posterior segment Development of new blood vessels in retinal leads to yellow deposits under the retina Risk factors are aging, smoking, high blood pressure, immobility	New treatment: juxtascleral injections of anecortave cortisone No real remedy to stop	[22,23]
Cataract	Lens is progressively transparent Alternation in α, β, γ crystalline protein Risk factors are ultraviolet light, diabetes, bad nutrition	Multifunctional antioxidants: curcumin, lanosterol, resveratrol Surgical removal of opaque lens	[24,25]
Conjunctivitis	The inflammation of conjunctival tissue Microbial infection can be the cause Noninfectious conditions are caused by allergens and irritants	Topical administration of antimicrobial and antiinflammatory agents	[26,27]
Diabetic retinopathy	Hyperglycemic disorders cause the upregulation of proinflammatory mediators, resulting in oxidative stress and inflammation of ocular blood vessel	Laser photocoagulation, vitrectomy Medications: intravitreous injection of corticosteroids, rabibizumab, and aflibercept	[28,29]
Fungal keratitis	Infection in traumatic cornea caused by a fungus	Oral or topical drug using corneal surgery	[30,31]

Continued

Table 2 Common ocular disease and agents for treatment—cont'd

Ocular disease	Characteristics and causes	Treatments	Refs.
	It could lead to impaired wound healing, corneal ulceration, stromal inflammatory Risk factors might be ocular or systemic disorders		
Glaucoma	Symptoms such as blurred vision progress to irreversible blindness because of the slow deterioration of the optic nerve axon and the death of retinal ganglion cells It develops as the result of oxidative and nitrative processes	Antiglaucoma drugs	[32,33]
Retinoblastoma	This is a kind of malignant tumor that usually appears in children younger than 5 years old. It is caused by a mutation in the tumor suppressor gene for the retinoblastoma protein	Radiotherapy, chemotherapy, surgery, cryotherapy	[34,35]

needles, which are directly injected through both the sclera and the vitreous humor. This method is invasive into all tissues and could cause intraocular infections [36]. At the early stage of MN use for ocular drug delivery, the hypothesis was that reducing the size and variety of needle morphology could prevent side effects, spare doses, and ensure injection to the correct site. Then, hollow MNs were studied for transferring drugs to the deeper tissue of the eye.

Hollow MNs have emerged as a promising option to treat ophthalmic disease. These are microsized, hollow needles that facilitate delivering active

agents through that hollow area to the injection site. The most reliable delivery of agents and nanoparticles into the suprachoroidal space (SCS) is achieved by hollow MNs 800–1000 μm in length with applied pressures of 250–300 kPa [37]. Triamcinolone was injected into the SCS by using a 33 gauge, 850 μm length hollow MN; it proved effective in reducing acute inflammation as a high-dose intravitreal injection [38]. A highly targeted, hollow MN was used to transfer the sulprostone and brimonidine antiglaucoma drugs to the supraciliary space in a rabbit model by using a 700–800 μm length. The results showed that the intraocular pressure was reduced by as much as 3 mmHg bilaterally in dose-related response while dose sparing was 100-fold compared to topical administration [39]. Gene delivery is challenging, especially in the scleral area, because of the complicated, complex anatomy and physiology of the eye. The gene delivery of an adeno-associated virus to the transscleral intravenous was facilitated by using a hollow MN; this was demonstrated in injections to nonhuman primates. This MN administration led to focal gene expression and minimal intraocular inflammation; moreover, the intravenous injection elicited greater systemic humoral immune response [40].

To increase the bioavailability of a drug into the eye by distributing the substances into the tissue and having them remain there, several studies were conducted to improve the spreading area of the drug. Suprachoroidal injections with bevacizumab and polymeric particles by MN in rabbit models remained primarily in the SCS and choroid for a period of months [41]. Another nanoparticle polymeric formulation was developed and injected to the SCS of the rabbit animals through a 750 μm length hollow MN. Adding hyaluronic acid in the formulation gave increased particle spread over the whole choroidal surface or localization adjacent to the ciliary body after administration [42]. After studying the localized and spreading ability of different structure particles, small molecules, and polymeric formulations through the suprachoroidal area after injection with a 750 μm length hollow MN, the data showed that particles encounter greater barriers to flow in the SCS compared to molecules. In addition, the colocalization of particles and polymeric formulations allow a larger area of fluorescent agent into the SCS [43].

The sustained release of drugs into the eye also has gained the attention of researchers because it could decrease the number of injections and increase patient compliance and permission. A hydrogel-based drug formulation has been developed for sustained delivery. Such a formulation has been applied with sunitinib-an antivascular endothelial growth factors for age-related

macular degeneration. The thermoresponsive hydrogel was injected through the porcine scleral model; it could offer the controlled release of sunitinib depending on the percentage of chitosan grafting [44]. A poloxamer formulation was delivered to the sclera area by using 400, 500, and 600 μm length hollow MNs; this application offered sustained release over 24 h, which varied with the insertion depth [45].

Coated MN: Recently, a self-plugging MN has been designed to plug and block a scleral hole with a swellable hydrogel at the bottom of the needle. Then, the coated material in the body of the needle was dissolved, delivering the drug into the vitreous humor. This platform facilitated only injection and sustained release through 7 days after injection. Notably, there was no difference in the arrangement of collagen fibers at the implantation site, no scleral thickness change, and no leakage of vitreous at the interface between the self-plugging MN and the scleral tissue of a pig eye. This demonstrated the successful application and delivery of the drug to the ocular tissue [46].

A pen-based MN is a device integrated with MN to facilitate mechanical strength while inserting and storing a single MN. A detachable tip pen-based MN was fabricated with the biodegradable material in the tip, and the active agent would dissolve with the dissolving agents. Such a structure successfully demonstrated the ability to transfer the fluorescent substance into the retina of beagle eyes [47]. Then, an octagonal, cone-shaped, 150 μm length poly(lactic-*co*-glycolic) acid polyhexamethylene biguanide tip MN was created, and the therapeutic applicability was tested in the cornea of a mouse model. Keratitis therapeutic efficacy was reported with the controlled release, and detachability was observed in a group of drug MN administration [48].

3.2 MN array for anterior segment administration

MN arrays are MNs that are fabricated with numerous needles on an array or base; the number of needles ranges from several to thousands and they are made of metal, silicon, and polymer. Such structures have been gaining research attention with the diversity of morphology and materials. Usually, these MN arrays are created in coated MNs, dissolving MNs, and hydrogel MNs. In terms of MNs for anterior area application, the MNs were designed as lens to apply on the cornea or a normal based-array.

The use of *contact lens* integrated with drugs is an interesting idea to deliver active substances directly to the cornea and treat the outer-layer

ophthalmic disease. Amphotericin B was loaded in polyvinyl alcohol (PVA) and polyvinyl pyrrolidone; a 5 × 5 pyramidal MN about 550 μm length was mounted at the center of the ocular lens, which proved to be an efficient therapy in a rabbit infected by *Candida albicans* [49]. Similar to that design, a pilocarpine MN was created and tested in ex vivo drug permeation in a porcine eye globe; this revealed significantly higher availability in the aqueous humor within 30 min of MN application compared with the solution formulation application [50]. The 550 μm length MN contact lens was loaded with a high molecular weight agent, cyclosporine A, which demonstrated the sufficient disposition of active ingredients into the excised porcine cornea. This MN application significantly enhanced the flux and retention of the drug when compared with commercial eye drops [51]. The sustained release ability of MN integrated with a soft contact lens was also reported with a silicon nanoneedle-loaded bevacizumab therapeutic ocular drug. The silicon MNs were fabricated at about 60 μm length, then the in vivo evaluation in a rabbit choroidal neovascularization (CNV) model was done. After one dose of treatment by an MN array, a rapid reduction in CNV occurred from days 1 to 7, followed by sustained, persistent, and continued reduction up to day 28 with nearly complete reduction while incomplete reduction was cited in the subconjunctival injection [52].

MNs array as a fluconazole microemulsion-loaded, two-layered dissolving MN was created with 3% chitosan and 20% PVA in a weight ratio of 1:4 as an outer layer while the inner layer was composed of the drug, eugenol, tween 80, PEG400, and water. This showed greater permeation into the excised porcine corneal tissue than the no-MN treated group and provided highly potential antifungal activity [53]. For the rapid release of the antifungal agent into the cornea, dissolving polymeric MNs loaded with besifloxacin in a 6 × 6 array format with needles of an average size of 961 ± 27 μm were used. The application of MN for 5 min significantly improved drug deposition and permeation through the cornea compared with the drug solution in an ex vivo test [54]. Polyvinyl pyrrolidone and hyaluronic acid dissolving MNs were also tested for corneal delivery with amphotericin B as the active ingredient [55]. Another MN platform with poly lactide acid and hyaluronic acid was fabricated and injected into a rabbit with no apparent ocular irritation. Moreover, in the animal treated by MN, a superior therapeutic effect was shown compared with a conventional eye drop formulation [56]. For the controlled release platform, self-implantable microdrug reservoirs containing an antiangiogenic monoclonal antibody were injected into the corneal neovascularization mouse disease model.

In the group of animals given a 600 μm length, 3 × 3 MN array, a reduction to 90% of the neovascular area was observed; this outcome had a synergistic therapeutic factor if quick-release diclofenac was applied after MN insertion [57].

3.3 MN array for posterior segment administration

To access the deeper structure of the ocular organ, conventional injections have been used (Fig. 1). Possible side effects include ocular tissue damage as well as infectious risks due to invasive techniques and repeated injections. Minimally invasive MN administration has been studied to prevent these adverse effects as well as increase bioavailability and even allow the controlled release of the drug. In terms of MN arrays for posterior chamber injection, dissolving MNs and coated MNs have been used frequently in studies.

Dissolving MNs: A triamcinolon acetonide nanosuspension MN could promote the transscleral penetration of hydrophobic molecules with a higher drug concentration observed in the deep layer of the sclera; the drug was deposited in an area 4.6-fold larger than plain drug-loaded MNs in Franz cell diffusion [58]. In a rabbit model, the triamcinolone concentration was greatest in the choroid-retinal complex and sclera after administration through intravitreal and MN application. Moreover, the MN injection provided a greater safety score compared to conventional injection [59]. A rapid, detachable MN using a porous water soluble layer was cited, as the MN tip almost immediately detached and embedded to the excised porcine sclera with the aid of impact insertion [60]. A high-molecular-weight drug has been tested for the ability to transfer to the posterior site by using an 800 μm length dissolving MN [61]. An ovalbumin polymeric MN demonstrated higher penetration to the posterior area of the eye compared with a needle-free patch [62]. The long-acting nanoparticle design of the ovalbumin MN could facilitate the sustained release of encapsulated proteins for more than 2 months, bypassing the scleral barrier [63]. A poly acrylic acid MN could allow for the matrix diffusion-controlled release of difluprednate to the posterior eye; the amount of drug penetration and retention in the sclera were decreased with an increase in the molecular weight of the polymer [64].

Hydrogel-forming MNs: This type of MN acts as a swellable needle that could deliver a drug to the injection site. Based on such properties, a hydrogel-forming MN was fabricated for the hypothesis that it could allow

for sustained release [65] and even interlocking control for easy extraction as the locking feature swells, securing the MN into the eyeball and letting the drug gradually diffuse [66,67].

4 Challenges and prospective

There have been numerous studies of MN as a minimally invasive technology for ocular drug delivery. Different types of MNs have been tested and successfully injected to different areas of the ocular organ. The results showed significant improvement in drug deposition to the right site as well as the controlled release of active substances. MNs have been designed in contact lens to fit the shape of the cornea and to overcome the anatomy features of such complicated tissue. In spite of achievement in researches, the application of a real MN array to treat the ophthalmic disease until the state-of-the art as there is no commercial array MN is used in clinic. There are also few ongoing clinical studies. The challenges that have hindered progress in this field include anatomical ocular barriers such as the cornea, tear film, anterior segment barriers, the sclera, Bruch's-choroid complex, and the blood-retinal barrier. Moreover, due to physical activities of the eye such as tear fluid speed, most of the drug concentration is lost due to nonproductive conjunctival absorption or systemic drainage; the physical force prevents foreign structure contact and the drug remains in the ocular tissue [5]. All the complex, complicated features that make up the eye also make it difficult to be invaded or controlled. However, MNs for ocular drug delivery have been gaining attention and increasing numbers of studies thanks to the development of diverse methods of fabricating MNs such as wet milling, drawing lithography, and micromolding in addition to the use of alternate materials such as polymers and hydrogels, which allow the creation of more MN platforms to take advantage of such structures and overcome barriers.

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CHAPTER 14

Potential of different types of microneedles in diagnosis and treatment of cancer

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Abbreviations

ADT	androgen deprivation therapy
APC	adenomatous polyposis coli
AuMSS	gold-core silica shell nanorods
BCC	basal cell carcinoma
CD4+	clusters of differentiation 4 cells
CD8+	cluster of differentiation 8 cells
EIC	electrical impulse chemotherapy
HA	hyaluronic acid
HPMC	hydroxypropylmethylcellulose
IgG	immunoglobulin G
MN	microneedle
MSN	mesoporous silica nanoparticles
PCL	polycaprolactone
PDT	photodynamic therapy
PGA	poly(glycolic acid)
PLA	polylactic acid
PLGA	poly(lactic-*co*-glycolic acid)
PLLA	poly-L-lactic acid
PVA	polyvinyl alcohol
PVP	polyvinylpyrrolidone
SPEIC	self-powered electrical impulse chemotherapy
TSCC	tongue squamous cell carcinoma

1 Introduction

The treatment chronology of cancer begins with oral administration of chemotherapeutic drugs to a gradual shift toward parenteral administration and a smooth transition to transdermal routes to increase the immune

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response, subjugate drug resistance issues, completely attenuate oral and parenteral discomforts caused to patients like frequency of injections, increased pain at the site of injection, hypersensitivity, thrombus formation, and difficulty in swallowing the unpalatable drugs [1]. The transdermal route extends its drawback with the presence of multistacked strata of cells termed as the stratum corneum. The plethora of techniques developed over time to improve drug penetration via skin are classified into three sectors: physical, chemical, and bio-chemical. The physical enhancers include ultrasound, iontophoresis, and thermal ablation; chemical modes of penetration cover the use of surfactants, solvents, liposomes, nanocarriers; bio-chemical methods encompass metabolic inhibition and incorporation of peptides [2,3]. The methods pose an obstacle with nerve damage due to physical methods and skin infections owed to the use of various chemical enhancers. The research on technological advancements in micromachinery led to the production of noninvasive, pain-less, microneedle (MN) technology that uses cone-shaped microsized needles to bypass the stratum corneum with the dimensions of 150–1500 μm length, base diameter of 250 μm and diameter of tip less than 10 upto 25 μm based on the fabrication method and material [4]. Nanoneedles (NNs) are comparatively small and do not damage the skin on repeated application, the drug penetration in NN is enhanced by magnetic and electric stimulus. The size of NNs is 5 μm in length and the tip diameter is 600 nm and is similarly classified into hollow, coated, solid, and polymeric NNs [5,6]. The application of MNs in cancer attributes to the high immunogenicity of the skin as resided by an exceedingly large number of antigen-presenting cells, consequently provoking an immune response via the activation of T cells [7,8]. The other potential advantages of MNs are the simultaneous loading of the drugs with prolonged controlled release, balancing the drug ratios to tackle drug-drug interactions [9–12]. The delivery of the chemotherapeutic drugs through the MNs significantly increases drug distribution and accumulation at the targeted site and reduces the possibility of drug leakage to the adjacent tissues thus attenuating the off-target effects with a better control of the therapeutic window. The MNs transfer the drugs into the systemic circulation by forming microchannels or micropores in the skin layer promoting enhanced permeation of micro- and macromolecules [1,4,13]. MNs show certain limitations owing to their dose loading, dissolving MNs are suitable only for drugs with low dose. The hollow and hydrogel-forming MNs can load high doses with increased frequency of administration resulting in skin reactions, inflammation, and infections at the site of administration including pain [14,15]. The discomfort of using MNs can also be weighed by the material of MNs used such as ceramic and glass.

MNs can be paired with immunotherapy, gene therapy, photothermal therapy, physical and chemical methods of permeation enhancers for an improved therapeutic action with a synergistic action and a controlled release pattern. MNs also explore applications in rheumatoid arthritis, cancer, antimalarial, diabetes, tetanus toxoid, etc. [16,17].

2 Materials used for manufacturing MNs

MNs are fabricated using a wide variety of inert materials possessing properties like good mechanical strength, biocompatibility, and inability to induce an immunogenic response, e.g., silicon, polymers like PLGA, PVP, PLA, PVA, and PGA, silica glass, borosilicate glass, metals such as titanium, stainless steel, platinum, and alloys, sugar and carbohydrates moieties like maltose, galactose, raffinose, trehalose, xylitol mannitol and ceramics of alumina, calcium sulfate dihydrate, and calcium phosphate dihydrate [18] (Table 1). The types of microneedles are represented in Fig. 1.

2.1 Solid microneedles

Solid MNs deliver the drug based on "poke and patch" approach and are fabricated from materials like stainless steel, PLA, PCL, PGA, PLGA, ceramics, etc. [19–21]. This approach utilizes solid nondrug-containing MNs to create temporary microsized channels to aid the permeation of conventional drug formulations like gel or ointment across the skin to give a local systemic effect [22]. Solid MNs are useful in enhancing the skin permeability and anticancer effect of many nanocarriers. A study utilized Dermaroller, a solid MN-based device with doxorubicin-celecoxib loaded liposomes for treatment of melanoma in a mouse model. Treating the skin with Dermaroller increased tumor inhibitory activity of doxorubicin-celecoxib over passive delivery routes by enhancing the skin permeability by twofold [23]. The "poke and patch" method using solid MNs was also used to treat the skin prior to breast cancer vaccination, which significantly raised the serum levels of immunoglobulins (IgG, IgG2a), and B cells (CD4+, CD8+) in the vaccinated animals resulting in fivefold enhancement of tumor suppressive activity in comparison with the unvaccinated group [24].

2.2 Hollow microneedles

Based on "poke and flow" approach, hollow MNs constitute an empty cavity filled with drug solution or dispersion and are manufactured using several

Table 1 Summary of materials and methods used for microneedle fabrication.

Material	Example	Types of microneedles	Method	References
Metals	Titanium, stainless steel, platinum, nickel, gold, and alloys	Hollow, solid, coated	Laser cutting, laser ablation, deep X-ray lithography, wet etching, metal electroplating	[35,38,51,52]
Ceramics	Alumina, calcium sulfate dihydrate, and calcium phosphate dihydrate	Hollow, solid, coated	Photolithography, deep X-ray lithography, micromolding	[19,53,54]
Polymers	PLGA, PVP, PLA, PVA, PGA	Solid, dissolving, coated, hollow, hydrogel forming	Photolithography, deep X-ray lithography, drawing lithography, micro-molding, melt casting, two-photon-polymerization	[34,55–58]
Silicon	Silicon	Hollow, solid, coated	Dry etching, wet etching, lithography	[59–61]
Glass	Silica glass, borosilicate glass	Hollow	Pulling pipettes	[62]
Sugars and carbohydrates	Galactose, raffinose, trehalose, xylitol, and mannitol	Dissolving	Micromolding, air blowing	[63,64]

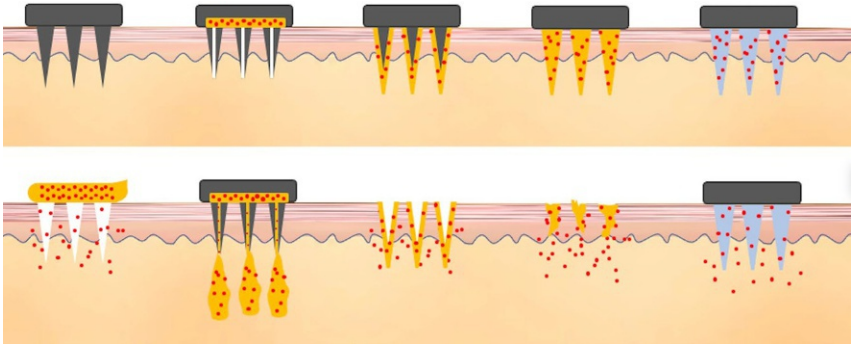

Fig. 1 Types of microneedles. *(Reprinted with permission from F.Y. Wang, Y. Chen, Y.Y. Huang, C.M. Cheng, Transdermal drug delivery systems for fighting common viral infectious diseases, Drug Deliv. Transl. Res. 11(4) (2021) 1498–508.)*

materials like glass, polymethyl methacrylate, stainless steel, etc. [25–27]. Unlike solid MNs, the drug administration and microchannel formation are achieved in one step. A hollow MN array utilized to administer Pluronic F-127 formulation of 5-fluorouracil peritoumorally resulted in a reduction of tumor volumes compared to the group treated with free 5-fluorouracil [28]. Hollow MNs also find use in the diagnostic application as evident by the study that utilizes a hollow MN-based point-of-care system [29]. Hollow MNs also provide excellent control over the quantity and time of drug delivery and rapid absorption of the drug than the subcutaneous route of administration [30]. However, hollow MNs show certain drawbacks such as they are expensive and complicated to manufacture, challenging to handle because of their delicate nature, and the potential for needle obstruction or leakage during insertion into the skin. Hollow MNs are more likely to suffer from structural damage, and to overcome this, beveled tip hollow MNs are made to include a constituent material other than silicon such as metals or polymers, which increases their hardness. Furthermore, the limitation of hollow MNs during tissue implantation due to clogging is overcome by side bore-opening hollow MNs [31,32].

2.3 Coated microneedles

Coated MNs also known as "coat and poke" approach involve coating the MNs with a drug solution that subsequently dissolves and deposits on to the skin layers [33]. Materials like PLLA, vinyl ether-maleic anhydride copolymer, PGA, etc., are used for manufacturing such MNs [34–36]. MNs coated with 5-fluorouracil, curcumin, and cisplatin utilized Soluplus, a copolymer

of polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol to improve the aqueous solubility of anticancer drugs and drug release profile [37]. In another example, doxorubicin-coated MNs were used for treating oral carcinoma. The MNs provided a minimally invasive, pain-free intratumoral drug delivery and showed uniform drug distribution up to a depth of 3 mm and minimum drug leakage [38]. Hollow MNs quickly deliver the drug to the skin in a single step but are limited by limited in terms of drug-carrying capacity and potential changes to the mechanical strength upon drug coating.

2.4 Dissolving microneedles

Using the "poke and dissolve" approach, MNs of such type dissolve completely after application on to the skin without leaving any sharp residues associated with MNs [33]. To manufacture such MNs, several water soluble/biodegradable polymers like PVA, PVP, dextran, dextrin, HPMC, etc., are used [39–41]. Dissolving MNs deliver exact doses of small molecular substances in a single step and are relatively inexpensive and straightforward to manufacture. The inclusion of drugs into the polymer matrix also enables tuning of the drug release pattern [42,43]. Drawbacks of dissolving MNs include low mechanical strength, irregular tip and MN shape due to improper manufacturing process, small dose delivery capacity, and skin irritation due to the presence of residual solvents [44,45]. A study described PVA-PVP MNs of doxorubicin and docetaxel for treating breast cancer. The mouse skin was easily penetrated by MNs leading to improved diffusion of drugs across the skin. Co-administration of doxorubicin and docetaxel resulted in a higher survival rate than intratumoral injections. Also, compared to the control group, mice given the drug-loaded MNs survived longer and had smaller tumors [46]. Similarly, cisplatin-loaded nanoparticles were embedded into dissolvable MNs of sodium carboxymethylcellulose, enabling the nanoparticles to pass the stratum corneum. The nanoparticle-loaded MNs substantially increased the cytotoxicity and apoptosis leading to reduce tumor volume and lower tumor weight with an apoptotic index of 58.6% [47].

2.5 Hydrogel-forming microneedles

A recent development based on "poke and swell" approach, hydrogel-forming MNs, is made of crosslinked super swelling polymers such as polyethylene glycol diacrylate, polymethyl vinyl ether/maleic acid-poly ethylene glycol, etc. Upon insertion into the skin, the hydrophilic polymers

absorb large amounts of water and swell up to release the loaded drug in a controlled manner [48,49]. Hydrogel-forming MNs are easy and cheap to manufacture, can be subject to gamma irradiation for sterilization, and are not limited in terms of loading capacity. Hydrogel-forming MNs find application in drug delivery as demonstrated by an example wherein metformin hydrochloride was delivered using this type of MNs in a sustained manner. The MNs possessed adequate mechanical strength and showed no signs of failure or fractures at a force of 36.3 N and significantly improved the permeation and bioavailability of the metformin hydrochloride ($12.94 \pm 2.96\%$ and $37.53 \pm 3.17\%$ for hydrogel-forming MNs compared to control at $0.45 \pm 0.52\%$ and $0.52 \pm 0.52\%$ at 6 and 24 h, respectively [49]). Similarly, hydrogel-forming MNs were used for delivering bevacizumab, a humanized monoclonal antibody used in treatment of metastatic breast cancer, metastatic colorectal cancer, advanced renal cell carcinoma, etc. The MN-assisted delivery of bevacizumab was found to be significantly more than the control group, i.e., without the assistance of MNs ($27.5 \pm 2.5\%$ for high dose reservoir group, $24.3 \pm 4.6\%$ for low dose reservoir group, and $0.9 \pm 0.8\%$ for the control group), thus demonstrated the efficacy and potential of hydrogel-forming MNs in the delivery of peptides and antibodies in the treatment of cancer [50].

3 Application of microneedles in cancer

MNs are presented for the localized or systemic administration of small molecule or macromolecular medications to address difficulties like drug leakage into adjacent healthy tissues, thereby diminishing the therapeutic safety and efficacy. Localized medication delivery by MNs can lead to significant tumor accumulation with minimal adverse effects and facilitate drug diffusion into deeper tumor areas. Additionally, cancer vaccines are intended for immune response generation in hosts assisting in the eradication of the targeted malignant tissue. Dendritic cells, antigen-presenting cells, and macrophages are primarily targeted for transdermal delivery of these vaccines via MNs. When adjuvants or antigens are introduced into this skin layer, dendritic and APCs cells are activated, stimulating the release of T (CD8+ and CD4+) and B cells by activated APCs generate an antitumor immune response [65]. Immune checkpoint regulators are crucial for maintaining immune homeostasis, safeguarding against tissue damage, and preventing the onset of autoimmune disorders. Multiple techniques are employed by cancer cells to disguise themselves and evade treatment and detection. Facilitating the use

of checkpoint inhibitors, immunotherapy attempts to avoid these maneuvers. Immune checkpoint inhibitors, namely cytotoxic T-lymphocyte associated antigen 4 (CTLA4), indoleamine 2,3-dioxygenase (IDO), and programmed cell death protein 1 (PD1) exert a regulatory influence on the effects of antibodies and T cells within tumor tissues. Additionally, various approaches utilizing MNs in delivering drugs, vaccine, or immunotherapy in cancer treatments are discussed in the following section (Table 2).

3.1 Breast cancer

Breast cancer is among the most common types of cancer in women and a major cause of death and morbidity worldwide. Approximately, 13% of women are diagnosed with breast cancer, with a death rate of 3% every year. It originates from the tissues in the breasts and spreads to the mammary gland ducts or the lobules that supply the ducts. If diagnosed early, it is a treatable form of cancer; but, if left untreated, the tumor becomes lethal due to its spread to other organs. Drug-loaded MNs can be used to deliver actives into the skin for facilitating antitumor action in cancer treatment [66].

MNs were used to deliver photosensitizer IR820 and cisplatin for synergistic breast cancer chemo-photothermal therapy. Polyvinyl-pyrrolidone vinyl acetate polymer was used to incorporate both agents. In vivo studies on mouse skin demonstrated a 50% release of IR820 and CDDP within 5 min of application. The combination successfully promoted ROS formation and activation of caspase 3 when exposed to laser (PDT), resulting in increased apoptosis and decreased cell proliferation. In vitro cytotoxicity studies on 4T1 breast cancer cell lines revealed reduced cytotoxicity and improved biocompatibility, implying the potential of MNs for synergistic photodynamic therapy for the treatment of breast cancer [66].

Another novel MN array loaded with hyaluronic acid (HA)-coated ribociclib (RB) transfersomes was developed for the treatment of HR+/HER2-metastatic breast cancer (MBC). This HA-PVA-PVP MN array was incorporated with HA-GMS-coated transfersomes for efficient CD44 receptor targeting. The MN array crafted tiny microchannels in the skin, allowing internalization of nanoparticles into tumor cells through systemic and lymphatic absorption by CD44 targeting with sixfold increase in the drug release. In vitro experiments demonstrated the potential of the MN array in effective eradication of MDA-MB-231 and LA-7 cells. The preclinical experiments on LA-7 mice also proved the effectiveness of the MNs in suppressing tumor growth up to 96% in breast cancer [67]. The efficacy of

Table 2 Application of MNs for delivery of actives in anticancer therapy.

Application	Microneedles	Therapeutic agents	Nanocarriers/ therapy	Cell line studies and animal model	References
Breast cancer	Vinylpyrrolidone—vinyl acetate copolymer (PVPVA)	Cisplatin	Photothermal therapy w/ IR820	4T1 breast tumor cell line/female Balb/c mice	[59]
Breast cancer	Zein	Tamoxifen and gemcitabine		MDA-MB-231 cells	[62]
Breast cancer	AdminPatch-1200	Murine breast cancer 4T07 whole cell lysate		4T07 breast tumor cell bearing Balb/c female mice	[64]
Breast cancer	Hyaluronic acid-polyvinyl acetate-polyvinyl pyrrolidone (HA-PVA-PVP)	Ribociclib	Transferosomes	MDA-MB-231 and LA-7 cells/LA-7 tumor-bearing mice	[60]
Breast cancer	Polyvinyl acetate	Artesunate	Solid lipid nanoparticles	MCF-7 cells	[61]
Breast cancer	AdminPatch-1200	Resveratrol	Nanostructured lipid carrier	MDA-MB-231 cells	[63]
Tongue squamous cell carcinoma	Hyaluronic acid	Curcumin	Curcumin nanodrugs	Cal-21 cells	[67]
Superficial skin tumors	Polyvinyl acetate/ polyvinyl pyrrolidone	Doxorubicin	IR820		[70]

Continued

Table 2 Application of MNs for delivery of actives in anticancer therapy—cont'd

Application	Microneedles	Therapeutic agents	Nanocarriers/ therapy	Cell line studies and animal model	References
Skin cancer	PEGylated gold nanorod-coated poly(L-lactide)	Docetaxel	MPEG-PDLLA micelles	A431 cells	[24]
Superficial skin tumors	Hyaluronic acid	Doxorubicin	Gold nanocages (AuNCs)	B16F10 cells/B16F10 induced C57 mice	[71]
Nodular basal cell carcinoma	PMVE/MA	Redaporfin		Athymic nude mice Crl:NU (NCr)-*Foxn1*^{nu}	[72]
Basal cell carcinoma	Hyaluronic acid	Imiquimod (R837)	Poloxamer 407 nanoparticles	Crl:CD(SD) rats	[73]
Superficial tumors	Polyvinyl alcohol, polyvinyl pyrrolidone, polycaprolactone	Doxorubicin hydrochloride	Lab_6 (lanthanum hexaboride)	Porcine cadaveric skin/4T1 breast tumors bearing SCID mice	[74]
Prostate cancer	Silicon	RALA Peptide	Prostate stem cell antigen	HEK-293 and DC 2.4 cells/C57BL/6 mice	[77]
Cervical cancer	Polyvinylpyrrolidone/ polyvinyl alcohol and chitosan	Doxorubicin	AuMSS nanorods	HeLa cells	[80]

Cervical cancer	Polyvinyl pyrrolidone	RALA-E6/E7	DNA nanoparticles	NCTC-929 fibroblast cells (ATCC)/ C57BL/6 mice	[81]
Cervical cancer	Polyvinyl alcohol	RALA/E6 and E7 pDNA		NCTC-929 cells/C57BL/6 mouse	[81]
Osteosarcoma	Polyvinyl pyrrolidone	Doxorubicin	Idocyanine green-loaded mesoporous silica nanoparticles	MG-63 cells/ MNNG cell-induced BALB/c mice	[83]

artesunate-loaded MNs against breast cancer cells was investigated via the transpapillary route. The author utilized layer-by-layer (LBL) technique to coat the artesunate-loaded solid lipid nanoparticles with chitosan and HA for CD44 receptor targeting. The MN array demonstrated better biocompatibility, enhanced cytotoxicity of artesunate against the MCF-7 cells, and the potential of delivering actives through the transpapillary route directly into the breast cancer cells [68].

Different MN loading strategies and skin permeation approaches can affect drug delivery in cancer cells. The fabrication of MNs using zein protein by micromolding approach for codelivery of gemcitabine and tamoxifen. The ex vivo studies exhibited that the coated MNs approach resulted in greater tamoxifen deposition, whereas the poke-and-patch method provided greater tamoxifen deposition. Collectively, the level of drug delivered by the coat-and-poke approach was high compared to the poke-and-patch approach as it revealed the excessive deposition of gemcitabine alone in the breast cells [69].

MNs assist in enhancing the half-life of the actives, and this was established by incorporating AdminPatch Array 1200 to facilitate the delivery of resveratrol, a potent anticancer drug with a shorter half-life, through continuous transmission in breast cancer-bearing mice. The MNs demonstrated an enhanced penetration through the skin with a very low amount of resveratrol retained by the MNs. The comparative study showed the efficiency of MNs in delivering drugs to the site was much higher than the oral route. The preclinical studies revealed a significant increase in pharmacokinetic parameters of resveratrol when administered via MNs and a higher localization in breast cells than that of the oral route [70].

Recently, the development of vaccine-loaded MNs has witnessed a rise in delivering therapeutics to the skin for cancer treatment. In this context, a breast cancer vaccine was prepared by utilizing murine 4T07 breast cancer whole cell lysate as an antigen associated with tumor. The spray drying technique was utilized to formulate the particulate vaccine and administered into the skin using AdminPatch 1200. Confocal Microscopy was used to examine the penetration ability of the particulate vaccine. The results disclosed the ability of MNs to penetrate the skin was deep enough ($50 \pm 10\,\mu m$) to reach the Langerhans cells of the skin pertaining to the ability to induce immune response and memory cell generation for anticancer agents. Furthermore, in vivo experiments on immunization demonstrated a more robust cellular and humoral response, characterized by increased levels of serum immunoglobulins IgG and IgG2a, as well as higher concentrations of B and T cells (CD4+ and CD8+) [24].

3.2 Oral cancer

Oral cancer, also known as carcinoma of the mouth cavity, is a prevalent kind of cancer. Surgical excision, along with radiotherapy and chemotherapy, is usually the first-line treatment for oral cancer. A second treatment is the systemic delivery of chemotherapeutic agents like doxorubicin. The failure to entirely cure cancer and recurring tumors necessitates the direct injection of anticancer drugs into the targeted tissue. While, in the treatment of squamous cell carcinoma (SCC), different approaches like intratumoral and intralesional chemotherapies have been employed. Although various treatment techniques demonstrate excellent therapeutic effectiveness in clinical investigations, the manner of intratumoral delivery still remains one of the major problems faced in this area [71].

Research involved the development of coated MNs as an alternative approach for conventional hypodermic needle due to its leakage from tumor site into circulation and the incapability of proper drug distribution. The study aimed to be a minimally invasive and direct delivery to cancer cells by coating the MNs with nanoencapsulated doxorubicin. The study exhibited the efficiency of coated MNs in providing sufficient doses and minimizing drug leakage in the cancer cells, resulting in increased apoptosis. The study revealed the potential drug-coated MNs hold in effectively delivering drugs at controlled and well-distributed locations in oral carcinomas.

The use of pulsed electric fields shows enhancement in the penetration of drugs into tumor cells, a technique commonly referred to as electrical impulse chemotherapy (EIC). In this context, a SPEIC (Self-Powered Electrical Impulse Chemotherapy) was developed for the treatment of oral squamous cell carcinoma (OSCC). The manufactured MN electrodes possess the ability to enhance the electric fields applied to the EIC using e-triboelectric nanogenerators (TENG). Such nanogenerators were able to produce electric impulses for enhancing the cellular uptake of cisplatin by the OSCC cells, resulting in a significant reduction in proliferation and cell viability. The ex vivo studies demonstrated the potential of SPEIC approach owing to the increased apoptosis, programmed cell death, and enhanced drug uptake without causing any significant damage to the healthy cells [72].

Similarly, a photothermal trigger agent IR820 was utilized in combination with HA MNs loaded with curcumin nanodrugs as a novel approach in the treatment of tongue squamous cell carcinoma (TSCC). The study showed the potential of curcumin-nanodrug-loaded HA MNs in penetrating and inducing apoptosis in TSCC cells, while the addition of IR820

displayed a significant increase in performance owing to its photothermal ability. The cytotoxicity studies exhibited a superior survival rate of 90% in curcumin nanodrug groups, while the IR820 MNs demonstrated an elite performance with a survival rate of only 0.04% in Cal-27 cells. The use of MNs provided a minimally invasive and targeted delivery method for curcumin, while IR820 enhanced the effectiveness of the treatment by inducing photothermal therapy and the combination of these therapies provided an effective and novel approach for the treatment of TSCC [73].

3.3 Skin cancer

Skin/cutaneous carcinoma represents one of the most prevalent forms of cancer worldwide, impacting millions of individuals. Skin cancer is classified into three major types based on the site of origin: SCC, melanoma, and basal cell carcinoma (BCC). Melanoma, the cancer of melanocytes, is considered the most dangerous of these cancer types and BCC is the most commonly occurring form of skin cancer. DNA damage caused by excessive exposure to UV radiation, free radicals, and reactive oxygen species is the primary cause of skin cancer [74]. Several conventional therapies are used over the past several years, but they fail to eradicate the disease, and the risk of relapse remains substantial. Numerous ways like intravenous injections, topical agents, transdermal patches, spray-formulations, and foam formulations are utilized as drug delivery methods in skin cancer treatment, such methods tend to have unsatisfactory results due to their limited bioavailability and targeting. Orally or parentally delivered drugs undergo first-pass metabolism or deposits at undesirable locations, resulting in cellular toxicity. Thus, research is undertaken on the use of MNs considering the benefits of subcutaneous targeted delivery [75].

An NIR-triggered two-stage MN loaded with doxorubicin and IR820 was fabricated for the treatment of superficial skin tumors (SSTs). The two-stage MNs consist of polycaprolactone and lauric acid tips loaded with doxorubicin and IR820 (Fig. 2). The base entailed phase-changing PVA/PVP, which dissolved owing to the absorption of body fluids after insertion, and keeping the arrowheads penetrated in the skin tissue. The activation of IR820 under NIR radiation triggered the arrowheads to release doxorubicin. The MNs embedded with doxorubicin and IR820 successfully inhibited the proliferation of melanoma cells owing to the synergistic PDT and chemotherapy [76]. Similarly, another study involved the synthesis of photosensitive PEGylated gold nanorod-coated poly(L-lactide) MN arrays

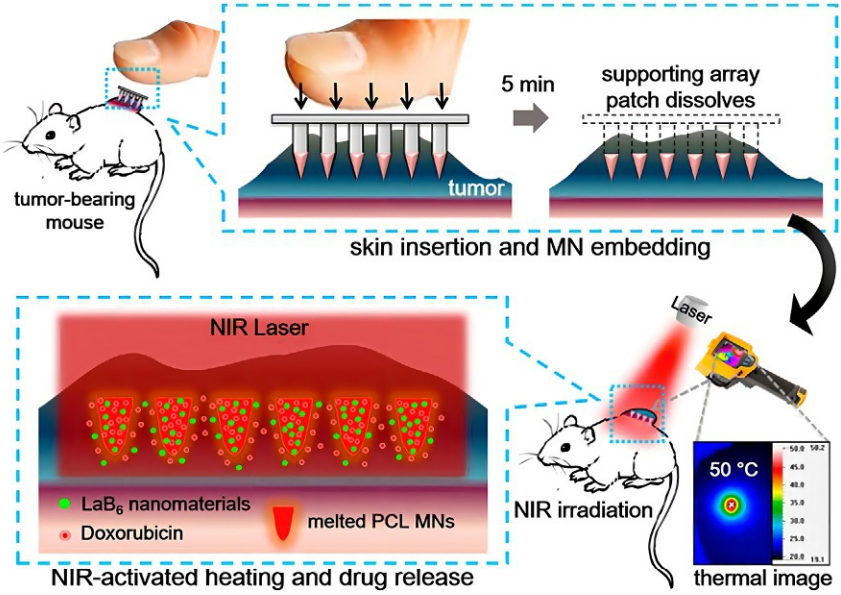

Fig. 2 Schematic illustration of microneedle-mediated chemo-photothermal therapy for the treatment of skin cancer. *(Reprinted with permission from M.C. Chen, Z.W. Lin, M.H. Ling., Near-infrared light-activatable microneedle system for treating superficial tumors by combination of chemotherapy and photothermal therapy. ACS Nano. 10(1) (2016) 93–101. https://doi.org/10.1021/acsnano.5b05043.)*

loaded with docetaxel-MPEG-PDLLA micelles for synergistic PDT and chemotherapy for the treatment of A431 cells. The MNs demonstrated excellent skin insertion properties and superior photothermal properties. A combination of docetaxel-MPEG-PDLLA micelles and PEGylated gold nanorod-coated poly(L-lactide) MNs was able to show superior synergy and completely eradicate the A431 tumor cells with no recurrence [77]. A similar approach was utilized for the incorporation of synergistic PDT and chemotherapy-based dissolving MNs loaded with gold nanocages (AuNC) carrying doxorubicin for treating SST. The AuNC not only improved the mechanical strength of the MNs but also showed effective transdermal delivery of doxorubicin due to its exceptional photothermal ability. The ex vivo results supported the fact that a synergistic approach involving chemotherapy combined with photothermal therapy not only demolished the SST but also diminished the adverse effects and displayed better-controlled inhibition on malignant cells than a single conventional therapy [78].

In major cases, conventional PDT is limited to the treatment of SSTs and fails to reach deep skin tumors like nodular BCC. MNs can facilitate deep skin delivery of actives via PDT without any unwanted adverse effects. One such study involved the use of Gantrez S-97 (PMVE/MA) dissolving MNs for delivery of (5,10,15,20-tetrakis[2,6-difluoro-3-*N*-methylsulfamoylphenyl] bacteriochlorin) (Redaporfin), a near-IR photosensitizer. The in vitro deposition studies illustrated the delivery of drug 5 mm down into the skin and in vivo studies in athymic nude mice Crl:NU(NCr)-Foxn1^{nu} displayed a burst release and localized drug delivery [79].

Various immunotherapies are extensively investigated the treatment of skin cancer. A study devised the use of amphiphilic block copolymer nanoparticles to incorporate imiquimod (R837) possessing the ability to suppress cancer cell proliferation by inhibiting angiogenesis. The Poloxamer 407 nanoparticles were coated with cancer cell membranes and loaded into dissolving MNs. The MNs were found to impede the growth of cancer cells during prophylactic vaccine immunotherapy and therapy. After 21 days, the tumor size decreased 8 times, proving the therapeutic efficacy of the dissolving MNs [80]. Another study incorporated cytotoxic T-cell epitope peptides (SIINFEKL) to inhibit the growth of the antigen-carrying tumor. HA MNs were used to deliver SIINFEKL for prophylactic immunotherapy. HA-conjugated peptide was loaded into dissolving HA MN. The MNs act as a transdermal vaccine carrier and retained the peptide in the skin for over 24 h and were able to significantly inhibit the tumor growth in B16 melanoma mice model by inducing antigen-specific cytotoxic responses [81].

3.4 Prostate cancer

Prostate cancer is the most prevalent type of noncutaneous malignancy and the second most common cancer in men, followed by lung cancer. In addition to hereditary and environmental variables, hormone abnormalities are the primary cause of chronic inflammation [82]. Current treatments for prostate cancer include surgical treatment or prostatectomy for the early stages of the disease, immunotherapy, androgen deprivation therapy (ADT), image-guided radiotherapy, or bisphosphonate therapy performed alone or in conjunction with techniques such as brachytherapy. Various chemotherapy drugs, such as abiraterone acetate, docetaxel, bicalutamide, nilutamide, degarelix, enzalutamide, and prednisolone, are approved for prostate cancer treatment. Prostate cancer, apart from the treatment with anticancer drugs, is also explored as a potential target for cancer vaccines like

PROSTVAC. Nevertheless, certain clinical trials involved with the DNA vaccine raised safety and efficacy concerns [83]. Therefore, MNs were utilized as an option to improve the efficacy of these cancer vaccines by improving the distribution of therapeutic components to the site of the localized tumor.

A study involved the investigation of a two-tier delivery system comprised of cationic RALA/pDNA nanoparticles into MNs for DNA vaccination against castrate-resistant prostate cancer. This study demonstrated the use RALA/pPSCA conjugate for mice immunization and tested the complexing ability of PSCA and NP-MN. The immunization with RALA/pPSCA-loaded MNs exhibited a tumor-specific immune response in both prophylactic and therapeutic prostate cancer models in vivo, depicting its ability to treat prostate cancer [84].

The use of ADT is reported for treating patients with localized or metastatic breast cancer. Studies describe the potential of luteinizing hormone-releasing hormone analogs (LHRHa) as one major kind of ADT used in the treatment of prostate cancer. Hence, one study exploited MNs to facilitate the transdermal LHRH analogs delivery for enhanced efficacy. The study utilized a dissolvable MN array synthesized with chitosan and PVA/PVP for controlled release of LHRHa to the skin layers. In vivo studies evaluated the efficacy of MNs, where the castration levels were maintained for about 14 days, suggesting the use of LHRHa-loaded chitosan MNs was able to effectively sustain the desired action and proved to be a useful method for ADT treatment [85].

3.5 Cervical cancer

Cervical carcinoma is the fourth most common type of cancer in women. It begins in the cells lining the cervix, the lowest portion of the uterus that links to the vagina. In its early stages, it is not lethal, but if left undetected, it can metastasis to other organs, including the colon, liver, urinary bladder, ovary, vagina, and rectum causing severe illness [86]. The majority of cervical cancer cases are associated with HPV (Human Papillomavirus) infection, with oncoproteins E6 and E7 responsible for pathogenesis. The gradual spread of infection can be treated in its early stages; however, persistent infection with different HPV strains can lead to cancer. For the successful treatment of cervical cancer, conventional cancer therapies and various revolutionary novel delivery techniques are currently under development.

A study involved the combination of photothermal and chemotherapy to enhance the tumor-killing capabilities and improve the efficacy of cancer

treatments. MNs were fabricated using PVP and coated with chitosan by electrospraying technique. These MNs were co-loaded with gold-core silica shell nanorods (AuMSS) and doxorubicin in a layered manner. The AuMSS nanorods facilitated dual action, where gold nanoparticles assisted in photothermal therapy and mesoporous silica coating provided colloidal stability and selective targeting. The MNs successfully penetrated the tumor cells, inducing a layer-dependent release of doxorubicin and demonstrating an outstanding combined chemo-photothermal effect against cervical cancer cells [87].

The oncoproteins E6 and E7 serve as target antigens in DNA-based-vaccine and are linked to the pathogenesis of cervical cancer. The use of MNs in the delivery of such a vaccine was proposed in a study wherein, a two-tier MN system was initiated, consisting of PVP base and DNA condensing peptide RALA in cationic nanoparticles for cutaneous delivery. The in vivo studies in NCTC-929 cells exhibited the richness of IgGs in the cells associated with RALA/E6-E7 NPs and displayed an enhanced TC1 cytotoxicity. Compared to intramuscular injections, the administration of RALA/E6-E7 MNs vaccine in mice resulted in a greater antigen-specific humoral immune response. RALA/E6-E7 MNs exhibited tumor suppression in mice, owing to their specificity compared to other routes [88]. Similarly, another study involved the use of lyophilization technique to enhance RALA/pDNA NP loading capacity in dissolving PVP MN patches. This process significantly increased the dose and allowed up to 50 μg pDNA per patch without causing any manufacturing defects in the MNs. In vivo tests performed in C57 BL/6 mice demonstrated excellent tumor suppression, decreased proliferation, and increased tumoral response. Hence, this study proved to be a novel fabrication process to increase the delivery rates of MNs compared to the traditional methods [88].

3.6 Osteosarcoma

Osteosarcoma is a common malignant bone tumor that most commonly affects children and teenagers under the age of 20, mainly on the surfaces of long bones. The rapid proliferation of osteosarcoma cells derived from bone-forming mesenchymal cells forms tumor bone-like and bone tissues directly or indirectly [89]. A combination of PDT and chemotherapy was utilized with dissolving MNs to ensure the simultaneous delivery of DOX and indocyanine green (ICG) to the tumor site to employ their synergistic effects in the treatment of osteosarcoma (Fig. 3). Mesoporous silica

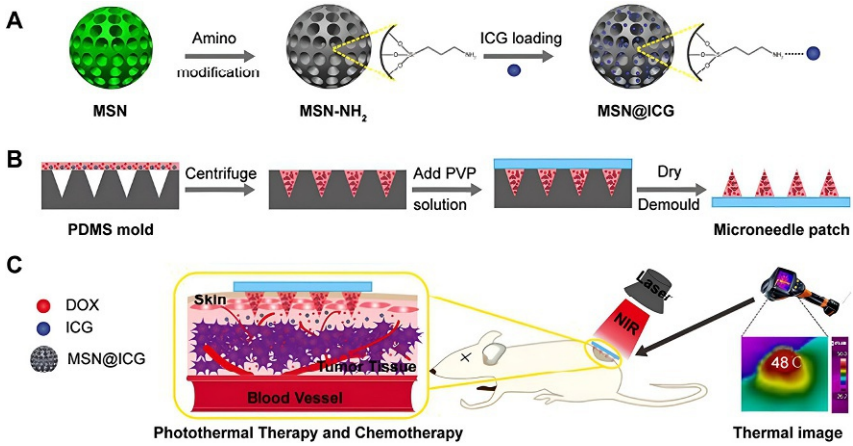

Fig. 3 Mesoporous silica nanoparticles mediated photothermal chemotherapy for treatment of osteosarcoma. *(Reprinted with permission from P. Pei, F. Yang, J. Liu, H. Hu, X. Du, N. Hanagata, et al., Composite-dissolving microneedle patches for chemotherapy and photothermal therapy in superficial tumor treatment. Biomater Sci. 6(6) (2018) 1414–1423, https://doi.org/10.1039/C8BM00005K.)*

nanoparticles (MSNs) were utilized to maintain the stability and efficiency of PDT therapy. The MNs demonstrated good skin permeation abilities and were dissolved by the interstitial fluid (ISF) to release DOX and ICG locally at the tumor site. The in vitro cytotoxicity studies in MG-63 human osteosarcoma cells exhibited maximum cell death when treated with a combination of DOX and PDT with a cell viability changing from 50% to 30% when treated with NIR irradiation for 2 min. In vivo studies on tumor-bearing rat model demonstrated a higher cell necrosis ratio and significantly reduced tumor volume in PVP@DOX/MSN@ICG treated group. Therefore, the composite-dissolving MNs proved to be a promising strategy for chemotherapy when combined with PDT for synergistic therapy of superficial tumors [90].

4 Cancer diagnosis

POC diagnostic devices are tiny portable systems or laboratory-scale equipment generated through advancements in lab-on-a-chip, downsizing, or microfluidic methods. POC devices can diminish the time spent in clinics and emergency departments as well as allow nonexperts to diagnose and receive the results. MN represent a state-of-the-art approach that has yielded novel insights and enabled prompt diagnosis of various types of cancer like

breast cancer, ovarian cancer, skin cancer, etc. Interestingly, MNs assist in the collection of ISF by utilizing quick skin piercing or stripping methods; the collected ISF can afterward be employed for accurate detection, offering an opportunity to obtain diagnostic information regarding cancer [91].

Polymeric MNs bear the potential to be utilized in cancer diagnosis due to their ability to "collect-and-detect" biological samples due to the distinctive skin penetration capability they hold in addition to cancer therapy. The continuous exposure to ISF allows the MNs to detect cancer by noninvasive fluid delivery to the channels carrying sensor [92]. For example, a study involved the development of tyrosinase (TYR) detecting MNs integrated with wearable electrochemical sensors for analysis of a pig model for cancer. TYR enzyme serves as a cancer biomarker and can be detected utilizing wearable electrochemical sensors by coupling catechol on the transducer. The flexible epidermal bandage was built with robust inks immune to mechanical deformation. Simultaneously, the hollow MNs were packed with catechol-coupled carbon paste for TYR level detection. The sensing devices were connected to a compact, wearable electronic chip for wireless transmission of data to any portable device. This electrochemical sensor was employed in the device owing to the painless and noninvasive skin monitoring ability displayed by the MN biosensor, holding a promising potential for decentralized melanoma screening [93]. Similarly, another study developed a novel complementary metal-oxide semiconductor (COMS) vascular endothelial growth factor (VEGF) electrochemical sensor for cancer diagnosis from human blood. The sensor incorporated peptide-aptamer-based gold-cloaked silicone MNs for the detection of electrochemical reactions with VEGF. The contact with PBS and blood serum, allowed the MN sensors to detect the cancer-indicating VEGF. The binding of VEGFs to the peptide-aptamer complex suggested the change of capacitance among the MNs. This unfolding of capacitance change between MNs allows the biosensor to detect VEGF within a range of 0.1 to 1000 pM and clear selectivity [94].

On a similar note, an electrochemical transdermal immunosensor was prepared for early diagnosis of breast cancer (Fig. 4). The study involved the fabrication of Au-coated silicone MN arrays as an electrochemical transducer and biomarker extraction platform for capturing ErbB2, a key biomarker in breast cancer. The surface-functionalized immunosensor was tested using a phantom gel mimicking the different layers of skin, where ErbB2 biomarker was extracted from the gel and analyzed using DPV. The linearity in the detection of biomarker proved the uniqueness of the

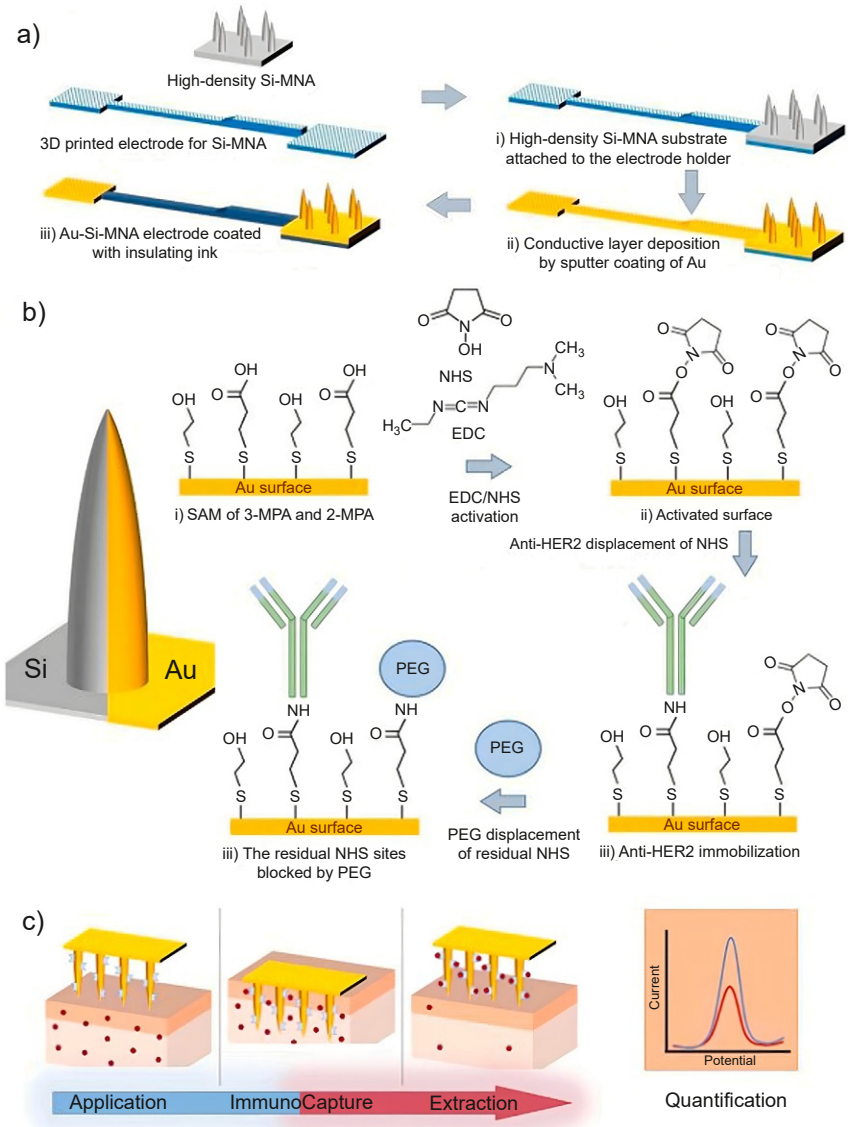

Fig. 4 Schematic illustrations of (A) electrode-based MN preparation, (B) anti-HER2 antibody conjugation and surface functionalization on Au-Si-MN array, and (C) MN-based immunosensor detection. *(Reprinted with permission from M. Dervisevic, M. Alba, T.E. Adams, B. Prieto-Simon, N.H. Voelcker, Electrochemical immunosensor for breast cancer biomarker detection using high-density silicon microneedle array, Biosens Bioelectron. 192 (2021) 113496. https://doi.org/10.1016/j.bios.2021.113496.)*

technique and displayed the potential it holds in the development of wearable POC devices [95].

A higher content of nitric oxide (NO) acts as a biomarker for cancer detection at the early stages of cancer. This process is supported by inducible NO synthase (iNOS) that contributes to angiogenesis leading to cancer progression. In this context, A MNs biosensor was incorporated into melanoma tissues for NO content analysis, exhibiting twofold increase in its concentration than the normal tissues. The in vivo application of hemin-functionalized MNs on melanoma-induced mouse skin revealed an electrical readout indicating a higher amount of NO in the melanoma cells than the control tissue [96]. Similarly, another study involved a polymeric MN array-based biosensor for endomicroscopic imaging and detection of NO in colon carcinoma. The biosensor comprised of PEDOT-coated and hemin functionalized (ferric chloride heme) polycaprolactone MNs. These MNs featured excellent carrier mobility, allowing direct, real-time, rapid, and uninterrupted readings of electrically NO signals with high sensitivity, stability, and selectivity. Furthermore, in vivo study demonstrated the use of MN biosensors in consonance with white light endomicroscopy, allowing tumor identification not only in the colon but for detecting cancer-specific overexpression of NO [96]. Another research work fabricated hydrogel MNs for early cancer detection by detecting tumor-specific exosomes. Herein, the MNs were fabricated using polycaprolactone, and coated with alginate hydrogel and glypican-1 (GPC-1) antibodies for effective targeting of tumor-specific exosomes. The hydrogel MNs expand when inserted, followed by absorption of the ISF from the skin, and the exosomes contained in tumor bind with GPC-1 antibodies inside the hydrogel. The obtained exosomes are separated further from the ISF to analyze the biomarkers related to the tumor. The in vivo experiments in colorectal cancer-induced mice demonstrated the early diagnosis of cancer using ELISA technique. This noninvasive diagnosis system utilizing extraction of exosomes samples proved to be an important step in early cancer diagnosis [97].

Another study involved the development of a wearable MN patch for simple and quick collection of Epstein Barr virus cell-free DNA (EBV Cf DNA) for monitoring circulating cancer DNA in the body. The fabrication involved an MN array RPA electrochemical flexible microfluidic device for quantification of EBV Cf DNA using POCT. In vivo studies revealed the maximum efficiency of EBV Cf DNA capture to be 93.6% and proved the implications the MNs hold for people suffering from Cf DNA-related disorders and cancer metastasis in a minimally invasive manner [98,99]. Taken

altogether MNs are promising devices for the extraction and detection of cancer biomarkers; however, in the future, MNs are recommended for individualized cancer diagnosis. Patients benefit from the ease of MNs-based diagnosis owing to its rapid and efficient health assessments rather than frequently seeking medical assistance for complicated and expensive medical diagnoses. However, further research is still required to determine their application.

5 Clinical studies

A plethora of research is carried out on the treatment and diagnosis of various diseases using MNs. There are about 64 clinical trial studies on MNs, out of which several clinical studies are working on the treatment of cancer [99].

A clinical study involved the use of doxorubicin-loaded dissolving MNs in phase I clinical trials. The goal of this study was to determine the safety and efficacy of utilizing an MN patch to treat cutaneous T-cell lymphoma (CTCL). This MN patch was placed in CTCL patients' skin plaques for skin cancer therapy. Another clinical trial aims to investigate the safety and feasibility of MN-mediated intratumoral delivery of SD-101, a TLR9 agonist, in combination with pembrolizumab in patients with advanced solid tumors. The study enrolled 18 participants who received SD-101 via MNs and pembrolizumab via intravenous infusion. The study's primary outcome measures the safety and tolerability of the treatment, while the secondary outcome measures include pharmacokinetics, pharmacodynamics, and overall response rate. Table 3 summarizes other clinical trials involving MN techniques in cancer therapy.

6 Regulatory aspects of microneedles

The MNs included under the category of medical devices as per the FDA are defined under the section 201 (h) of the FD&C Act, 21 U.S.C. and 321 (h), classified under special control class II under section 513(f)(2) of the FD&C Act with the certitude of safety and effectiveness of the microneedling device along with patient enhancement [100]. A company needs to submit premarket submission 510(K) equivalent to section 513(i)(1)(A) FD&C Act of the legally marketed device and register their device under 21 CFR 878.4430 to the FDA to prove the safety and efficacy of the device before bringing it to the market [101,102]. The center for devices and radiological health (CDRH) sector of the FDA regulates the manufacturer, repackages, and importers of medical devices in the United States. The classification of

Table 3 Status of clinical trials based on MN technique in cancer.

Sr. no.	Clinical trial number	Condition	Drug	Phase	Status
1	NCT04928222	Basal cell carcinoma	Doxorubicin	Phase 1 Phase 2	Recruiting
2	NCT05377905	Squamous cell carcinoma	Doxorubicin	Phase 1 Phase 2	Not yet recruiting
3	NCT05329532	Triple negative breast cancer Renal cell cancer Ovarian serous adenocarcinoma Squamous cell carcinoma	Pembrolizumab	Phase 1 Phase 2	Recruiting
4	NCT03646188	Basal cell carcinoma	Doxorubicin	Phase 1	Terminated
5	NCT04249115	Benign skin tumor	Nano-pulse simulation	–	Completed
6	NCT02192021	Cutaneous T-cell lymphoma	Doxorubicin	Phase 1	Recruiting
7	NCT04730583	Cutaneous neurofibroma	Kybella	Phase 1	Recruiting

medical devices falls under three categories: exempt Class I (low to medium risk), Class II (medium to high risk) also known as the 510(k), and premarket approval Class III (high risk) [103]. The class I MNs are required to be less than or equal to 0.3 mm and should not disrupt the anatomy and function of the skin [104]. FDA defines MN devices that treat wrinkles; deep scars left by skin burns, acne; stretch marks, alopecia, dermatoses, wound healing, collagen production, and angiogenesis. It also takes into consideration the length, sharpness, and degree of motorized or manual control to penetrate the deep layers of skin to show the desired effect and not just a surface phenomenon that claims improvement in the skin glow, and smoothness without entering the living layers of the skin [100,105]. The devices under class III require premarket approval due to the high possibility of serious injury to the patient, thus requiring submission of a clinical database to justify the labeled claim, safety, and effectiveness of the MN device [103,106]. The "special control" regulatory requirements encompass class II devices with the inclusion of performance standards, registries of patients, special labels, premarket data requirements, and postmarketing surveillance [103]. The necessary parameters are to verify and assure the safety and effectiveness of the device to be marketed. The 510 section of the general controls covers the listing of devices and registration, premarket application (510K), and reprocessed devices that are for single use [107]. The FDA now encourages eCopy also termed as electronic submission template and resource (eSTAR) submissions of premarket devices through the CDRH portal and by the start of October 1, 2023 all the 510 (k) submissions (unless exempted) must submit applications using eSTAR [101]. The positioning of MNs in the regulations is still abrupt and unsettled, under the FDA. The dissolving MNs are regulated as a device/drug combination product and under the European Union dissolvable MNs are categorized under the medicinal product [15]. MNs are required to address sterility issues, ease of use, packaging, safety, and disposal systems. The regulatory agencies mandate MNs to qualify standard pharmacopeial test for endotoxin for its sterility and many manufacturers do not tend to shift their product line to MNs from parenteral as aseptic manufacturing of MNs will be very expensive [15]. The sterility of MNs using gamma radiation, and heat could reduce the drug potency by causing drug and carrier damage and can also contaminate the MN system [108]. The regulatory requirements for clinical use also demand several other parameters such as uniformity of content for the system design, packaging to avoid microbial contamination of polymeric MNs and accidental piercing, disposing of systems as MN materials made of nonbiodegradable materials

and nondissolvable materials pose a threat to the environment, the safety of the MNs with respect to patients as polymeric MNs such as dissolving type can deposit on the skin on prolonged use and cause infection, formation of granuloma at the site of application and thus requires assurance of immunological safety validation as skin is a highly immunological site and can cause an immunological response on repeated MN piercings [109,110]. These potential obstacles can occur to scale-up the manufacturing of MNs with combinations of drugs. The regulations also recommend QC tests (ICH Q6 A) to meet the basic requirements of the MNs, different types of MN will dictate a separate QC test [111]. Generally, MNs need to pierce the skin, enhanced drug penetration, and need to be removed intact postusage. On the principles of the general considerations of the MNs the dissolving MNs are required to completely release the drug as per the type of release required, hollow MNs need to be open for the entire duration of the therapy time, and the hydrogel-based systems need to swell for the drug to release to entire the systemic circulation. The QC tests are dissolution, friability, mechanical testing like needle hardness, MN bending, fracture, insertion, disintegration, drug uniformity, stability, sterility, water content, microbial limits, antimicrobial preservatives, osmolarity, and functioning capability of the system [109].

7 Safety of microneedles

MNs are safe as they avoid disruption of the skin structure and functions; however, the microchannels formed by the MNs can cause infection at the site of application due to bacterial entry. A research study by Gupta et al. compared the healing time of skin with the application of hypodermic needles and MNs, and it was found that skin treated with MNs healed faster. It is also stated that an increase in MN length and number of MNs results in increased pain majorly for the length of MNs and many other research conducted proved that needle length is the primary reason for pain [20]. The solid MN made of a metal length of 2 mm reported the highest visual analog scale (VAS) value of 4.70 ± 1.08, stainless steel MNs of the length 480/700/960/1450 μm showed a VAS of $2 \pm 2/5 \pm 3/10 \pm 9/15 \pm 17$. The coated MNs of silicon with a length of 250 μm confirmed a VAS of 0.5 (out of 10), and dissolvable MNs of hydrogel measured the length to 280 μm with a VAS value of 0.92 (out of 2). Dissolvable MNs of HA length

of 300/500/800 μm documented VAS value approximately equal to 15, 17, 18 (out of 100). The pain value based on VAS for hollow MNs made of silicon crystal of Length 450 μm was 34 and for length 600 μm the VAS value was 3.4 ± 1.7 (out of 10). For hollow MNs of glass length 500/750/1000/4000 μm, the insertion pain was 4/2/3/16 (out of 100) and the fluid infusion pain increased with insertion depth [112].

Skin irritation and inflammation are other safety concerns based on the type of drug loaded, density of MN bases, needle length, and deposition of polymer in the microchannels resulting in blockage. The tests to scale skin irritation are Draize dermal scoring criteria [113], trans epidermal water loss [13] for figuring the extent of skin damage, and Tristimulus Colorimetry Technique to quantify the erythema. Research conducted on cisplatin and IR80-loaded MNs displayed normal cellular morphology and hair follicles of the skin upon MN application, with no changes in the pathophysiology of the skin. The synergistic chemotherapeutic treatment of CDDP and IR820 in breast cancer demonstrated no safety concerns [66]. The gemcitabine encapsulation in MNs for pancreatic cancer proved the safety of using hematoxylin-eosin staining [114]. The results of a research study conducted on mice to evaluate the safety of HA MNs for a time period of 3 months reported no inflammation and erythema at the site of application, there were no abnormalities in the histopathology of the skin in 90 days [115]. These studies indicated the dissolving MNs on repeated application prevent damage to the skin and are safe to use. Overall, analyzing the results of several research verifies the claim that MNs have a big magnitude of safety as compared to other dosage forms.

8 Future perspective and conclusion

MNs are explored in the treatment and diagnosis of cancer and explore the potential to be a more suitable approach over the current cancer therapies under investigation. There are several research studies conducted on cancer with the use of MNs, the recent ones been combination therapy, gene therapy, and immunotherapy [4]. The combination of doxorubicin and docetaxel in PVP-PVA MNs for the treatment of breast cancer proved to be more efficient than monotherapy [46]. Another study formulated self-assembled nanodissolving MNs combined with phototherapy for melanoma cancer therapy [116]. The gold nanorod coated with poly(L-lactide) MN

improved the antitumor efficacy of docetaxel-loaded MPEG-PDLLA micelle to treat A431 tumor [77]. Under gene therapy, MNs based on PVA-PVP were loaded with E6/E7 pDNA RALA nanoparticles to establish therapy for cervical cancer [88]. Immunotherapy presents an innovative approach to load encapsulated DNA vaccines in MNs to protect vaccines, retain their stability, improve half-life, and being able to generate an extended response, in human lung carcinoma [4,117].

Even with numerous researches conducted on MNs for cancer, it is of prime importance to understand that the safety of microneedling devices along with preclinical and clinical investigations need to be practiced more for further development of novel ideas to build MN potential in the treatment of cancer. It is also necessary that the manufacturers follow the pharmacopeial requirements, GMPs, Quality control tests, sterility, packaging, disposal, and several other patient safety and acceptability parameters. MNs in cancer is a customized, personalized, and precision-based system for optimal patient safety. Theranostics is the new future for MNs that would enable minimal-invasive scanning, and monitoring of the disease and will also serve as a drug delivery system. The use of MNs in cancer can be stepped up by using biosensor-based MN smart systems to enable real-time data on the cloud and also receive personalized treatment via telemedicine [118,119]. The biomaterials used in biosensor-based MN systems can be designed sensitive toward temperature, pH, and magnetic fields to enhance the diagnostic features and aid in cancer therapy by continuous monitoring of drug, delivered, drug metabolites, targets, electrolytes, and the patient performance [120,121]. Internet of things in health care such as blockchain technology, RFID, can make data more accessible with easy traceability [122–124]. These unique strategies if imposed on MNs can be a breakthrough in the field of treatment and diagnosis of cancer.

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CHAPTER 15

Microneedle-mediated photodynamic/immunotherapy

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1 Introduction

Microneedle-mediated photodynamic/immunotherapy (MNPDI) is a novel therapeutic approach that has gained significant attention in recent years due to its potential to provide targeted and personalized treatment for various diseases. This approach combines two distinct therapeutic modalities, namely photodynamic therapy (PDT) and immunotherapy, using microneedles as a delivery platform [1].

PDT is a noninvasive procedure in which a photosensitizer is administered. When triggered by light of a certain wavelength, the photosensitizer produces reactive oxygen species (ROS), which can specifically kill malignant or diseased cells [2]. On the other hand, immunotherapy is a type of treatment that helps boost the body's immune system to combat cancer or infectious diseases by utilizing various approaches, such as monoclonal antibodies, vaccines, and adoptive cell transfer [3].

MNPDI is based on the premise that combining these two modalities can enhance the efficacy of treatment, especially for diseases that are difficult to treat using conventional therapies. The microneedles used in MNPDI are typically less than a millimeter in length and are made of biocompatible materials such as silicon or metal. They are designed to penetrate the skin and deliver the therapeutic agents to the target site, thereby minimizing

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systemic toxicity and improving therapeutic outcomes. It has several advantages over traditional therapeutic approaches. First, it can provide a localized and targeted delivery of therapeutic agents to the site of the disease, thereby minimizing systemic side effects. Second, it can enhance the efficacy of the treatment by combining two different modalities that have complementary mechanisms of action. Third, it can potentially overcome the limitations of traditional PDT and immunotherapy approaches, such as low selectivity, limited penetration, and low response rates. It has shown promising results in preclinical studies for the treatment of various diseases, such as cancer, bacterial and viral infections, and autoimmune diseases. For example, MNPDI has been shown to enhance the efficacy of PDT in the treatment of breast cancer, reduce bacterial load in skin infections, and enhance the immune response to viral infections such as influenza [4,5].

2 Microneedles

Microneedles are microscopic devices that have drawn a lot of interest recently due to their potential uses in a number of industries, including tissue engineering, drug delivery, and diagnostics. These devices are typically less than a millimeter in length and can be made of different materials such as silicon, metal, or polymer. Microneedles are designed to penetrate the skin or other biological tissues and deliver therapeutic agents, sensors, or other materials to the target site. They offer several advantages over traditional needle-based delivery systems, including painless administration, minimal trauma, and improved patient compliance. Microneedles are a rapidly evolving technology with exciting potential for improving healthcare and advancing scientific research [6,7].

2.1 Types of microneedles

Microneedles can be made of a variety of materials, including metals, polymers, and ceramics. They can be classified into several types based on their shape, size, and mechanism of action. Fig. 1 shows a few types of microneedles and their drug delivery methods. These include [8]:

- Solid microneedles: These are the most basic types of microneedles and consist of a single shaft without any internal channels. Solid microneedles are typically used for transdermal drug delivery, where they can penetrate the stratum corneum, the outermost layer of the skin, and deliver drugs to the underlying tissues.
- Hollow microneedles: These microneedles have one or more internal channels that can deliver drugs, vaccines, or other materials directly to the target site. Hollow microneedles are used for intradermal,

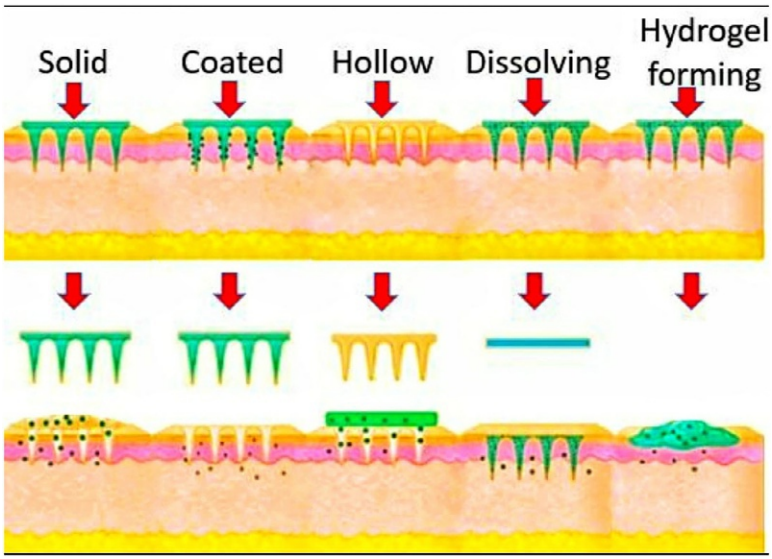

Fig. 1 Types of microneedles and their drug delivery methods.

subcutaneous, or intramuscular injections, and they offer several advantages over traditional needles, including painless administration, reduced tissue damage, and improved therapeutic outcomes [9].

- Coated microneedles: These microneedles are coated with a layer of drug or vaccine that dissolves slowly after insertion into the skin. Coated microneedles are used for transdermal drug delivery and can provide a sustained release of the drug over time.
- Dissolving microneedles: These microneedles are made of water-soluble materials such as sugars, polymers, or proteins, and they dissolve upon insertion into the skin. Dissolving microneedles are used for transdermal drug delivery, vaccines, or diagnostic tests, and they eliminate the need for needle disposal.
- Hollow metal microneedles: These microneedles are made of metals, such as titanium or stainless steel, and have one or more internal channels that can deliver drugs or other materials. Hollow metal microneedles are used for intradermal, subcutaneous, or intramuscular injections, and they can penetrate the skin with less force than solid needles.
- Microneedle arrays: These are arrays of microneedles, typically ranging from a few to hundreds, that can be used for large-scale transdermal drug delivery, vaccines, or diagnostics. Microneedle arrays can penetrate the skin simultaneously, and they offer high throughput and scalability for manufacturing [10,11].

2.2 Advantages of microneedles over conventional methods

Microneedles are a promising technology that offers several advantages over conventional methods and therapies. One of the most significant benefits of microneedles is painless administration. Microneedles penetrate the skin with minimal force, resulting in little to no pain or discomfort during administration. This is particularly important for patients who require frequent injections or treatments, such as diabetics or cancer patients. Improved patient compliance is another advantage of microneedles. Because microneedles are painless and easy to use, patients are more likely to comply with their treatment regimen. This can lead to better therapeutic outcomes and reduced healthcare costs [12,13].

Microneedles also reduce tissue damage compared to traditional needles. Traditional needles can cause tissue damage and inflammation, leading to pain, swelling, and other side effects. Microneedles, on the other hand, are designed to minimize tissue damage and reduce the risk of infection [14]. Moreover, microneedles can be designed to deliver drugs, vaccines, or other materials directly to the target site, such as the skin, muscle, or bloodstream. This can increase the efficiency and effectiveness of the treatment while minimizing side effects [15].

Another significant advantage of microneedles is sustained release. Some types of microneedles, such as coated or dissolving microneedles, can provide sustained release of drugs over an extended period of time. This eliminates the need for frequent injections or treatments and can improve patient comfort and compliance. Moreover, microneedles can stabilize unstable drugs and vaccines, improving their shelf-life and reducing the need for special handling [16].

Finally, microneedles generate less needle waste compared to traditional needles. Traditional needles are disposable and generate a significant amount of needle waste. Microneedles, on the other hand, can be designed to be reusable or biodegradable, reducing the environmental impact of needle waste.

2.3 Challenges faced by microneedles

While microneedles offer several advantages over conventional methods and therapies, they also face some limitations and challenges.

One of the main limitations of microneedles is their size. Microneedles are, by definition, very small, which limits the amount of material that can be delivered through them. This can be a challenge for drugs or vaccines that require large doses or for treatments that need to be delivered to deep tissues [11].

Another challenge faced by microneedles is the variability in skin thickness and density across different populations. This variability can make it difficult to design microneedles that can penetrate the skin effectively in all patients. Furthermore, skin conditions such as eczema, psoriasis, and scars can also affect the performance of microneedles. Moreover, the manufacturing of microneedles can be challenging and expensive due to the need for high-precision fabrication techniques and the use of specialized materials. The development of microneedles that can be mass-produced at a reasonable cost remains a significant challenge. Another limitation is the lack of standardized regulations for microneedle products. The lack of guidelines or standards can create uncertainty around the safety and efficacy of microneedle-based products, which can hinder their development and commercialization [14].

While microneedles offer several advantages over conventional needles, they are not suitable for all types of drugs, vaccines, or materials. For example, some drugs or vaccines may be too large or too sensitive to be delivered via microneedles. They also face limitations and challenges such as variability in skin thickness, high cost of manufacturing, lack of regulations, and suitability of materials. Addressing these challenges will be crucial for the widespread adoption of microneedles in clinical practice [17].

3 Photodynamic therapy

A photosensitizing chemical and a light source are used in photodynamic therapy (PDT), a medical procedure to kill cancer cells and treat other illnesses. PDT has received FDA approval to treat a number of malignancies, including skin, lung, and bladder cancer, as well as for the treatment of certain noncancerous conditions, such as acne, psoriasis, and age-related macular degeneration [18,19].

PDT works by first administering a photosensitizing agent to the patient. These agents are designed to accumulate in cancer cells and other target cells, such as bacteria, viruses, and fungi. Once the agent has been absorbed, a light source is then used to activate the agent, which generates ROS that destroy the target cells. The light source used in PDT can be a laser, light-emitting diode (LED), or other light sources, depending on the type and location of the target cells [19,20].

The photosensitizing agents used in PDT can be administered orally, topically, or intravenously, depending on the medical condition being treated. Some commonly used photosensitizing agents include ALA

(5-aminolevulinic acid) and MAL (methyl aminolevulinate). These agents are designed to selectively accumulate in target cells while minimizing absorption by healthy tissues. After administration of the photosensitizing agent, the patient must wait for a period of time before the light source is applied. This waiting period is known as the drug-to-light interval and is determined by the type of photosensitizing agent used and the location and type of cancer or other medical condition being treated. During this waiting period, the photosensitizing agent is metabolized and accumulates in the target cells, which allows for selective destruction of the target cells during the subsequent light exposure [20,21].

The light source used in PDT must be carefully selected to match the absorption spectrum of the photosensitizing agent being used. For example, the light source used for treating skin cancer may have a different wavelength than the one used for treating bladder cancer. The light source is then applied to the target area for a specific period of time, which can range from minutes to hours, depending on the type and location of the target cells [22].

PDT has several advantages over traditional cancer treatments such as surgery, radiation therapy, and chemotherapy. One of the main advantages of PDT is its selectivity, which allows for precise targeting of cancer cells and other target cells while minimizing damage to healthy tissues. Moreover, PDT can be repeated multiple times with minimal side effects, making it suitable for long-term treatment of chronic conditions [23].

However, PDT also has some limitations and side effects. One of the main limitations of PDT is the limited depth of tissue penetration, which can limit its effectiveness in treating deep-seated tumors. PDT can also cause some side effects, such as redness, swelling, pain, and blistering, which can last for several days after treatment. In rare cases, PDT can cause more serious side effects, such as scarring, infection, and vision loss [18].

3.1 Mechanism of action

Photodynamic therapy (PDT) is a medical treatment that involves the use of a photosensitizing agent, a light source, and oxygen to destroy cancer cells and other abnormal cells. The mechanism of action of PDT involves a series of complex biochemical and physiological events that ultimately result in the death of targeted cells. The first step in PDT is the administration of a photosensitizing agent, which is typically a chemical compound that has a high affinity for certain cells or tissues. These agents are usually administered

either intravenously or topically and can accumulate selectively in abnormal cells, such as cancer cells or bacteria, due to their enhanced uptake or retention capacity [24].

Once the photosensitizer has accumulated in the targeted cells, it is then activated by exposure to light of a specific wavelength. This light is typically delivered using a laser or other light-emitting device and is usually applied directly to the site of the lesion or tumor. The interaction between the photosensitizer and the light results in the production of ROS, such as singlet oxygen and superoxide, within the targeted cells. These ROS cause damage to cell membranes, proteins, and DNA, leading to cellular dysfunction and, ultimately, cell death [25].

The cellular response to PDT varies depending on the type of cell, the photosensitizer used, and the light dose delivered. In cancer cells, PDT can induce apoptosis or necrosis (programmed cell death and cell death due to injury, respectively) through the activation of specific signaling pathways. In addition, PDT can also stimulate the immune system to recognize and eliminate cancer cells, leading to long-term antitumor effects [26]. Fig. 2 shows its mechanism of action on tumors.

3.2 Advantages of PDT over conventional therapies

Photodynamic therapy (PDT) is a medical treatment that uses a photosensitizing agent, a light source, and oxygen to destroy cancer cells and other abnormal cells. Compared to conventional therapies, such as surgery,

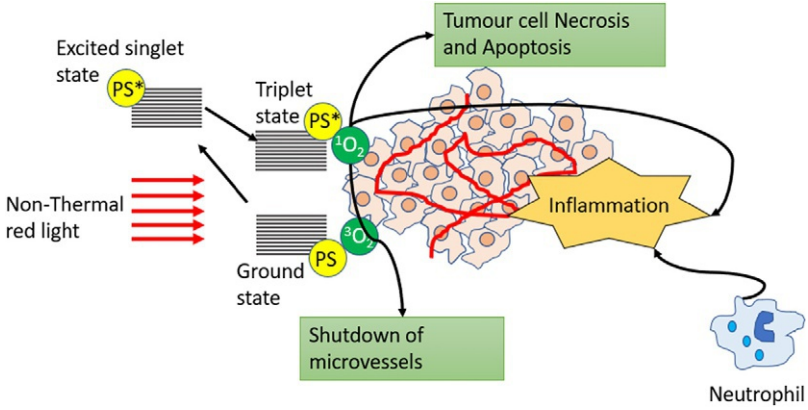

Fig. 2 The mechanism of action on tumors in photodynamic therapy.

chemotherapy, and radiation therapy, PDT offers several advantages that make it an attractive treatment option for certain types of cancers and other conditions.

One of the primary advantages of PDT is its ability to selectively target abnormal cells while sparing healthy tissue. The photosensitizer can be administered systemically or topically and can accumulate selectively in cancer cells or other abnormal cells due to their enhanced uptake or retention capacity. This allows for a highly localized and precise treatment of the affected tissue, minimizing damage to the surrounding healthy tissues and reducing side effects [27–29].

Another advantage of PDT is its minimally invasive nature. Unlike surgery, which often requires general anesthesia and can result in significant postoperative pain and recovery time, PDT can be performed using local anesthesia and typically results in minimal pain or discomfort. Moreover, PDT does not require the use of invasive instruments or incisions, thus reducing the risk of complications and infections [30,31].

PDT also has minimal systemic toxicity, unlike chemotherapy, which can cause severe side effects due to its systemic toxicity. The photosensitizer is generally metabolized and eliminated from the body within a few days, and the light source is only applied to the affected area, minimizing the exposure of healthy tissues to the treatment [32–34].

PDT can also be used in combination with other therapies, such as surgery and chemotherapy, to enhance their efficacy. For example, PDT can be used to shrink a tumor before surgery, making it easier to remove, or it can be used to sensitize cancer cells to chemotherapy, making them more susceptible to the effects of the drugs [27,35].

Finally, PDT is a repeatable treatment that can be performed multiple times, allowing for continued treatment of recurrent or residual cancer cells. Unlike radiation therapy, which often has a maximum cumulative dose limit due to the risk of healthy tissue damage, PDT can be repeated without significant risk of cumulative toxicity [28,33].

3.3 Clinical applications of PDT in cancer and other diseases

PDT has shown promise as a cancer treatment for several types of cancers, such as skin, lung, bladder, esophageal, and neck cancers. It is often used to treat superficial tumors, such as basal cell carcinoma, actinic keratosis, and squamous cell carcinoma. PDT has been particularly effective in treating early-stage nonsmall cell lung cancer and superficial bladder tumors. It has

been approved by the FDA for the treatment of certain types of AMD (age-related macular degeneration), a frequent reason for eyesight loss in older people. PDT can selectively destroy abnormal blood vessels located under the retina, thus reducing the risk of vision loss [28,36,37].

PDT has also been investigated as a treatment option for a chronic skin condition that causes red, scaly patches on the skin called psoriasis. PDT can reduce inflammation and normalize skin cell growth. In addition, PDT has shown potential as a treatment option for various infections, including bacterial, viral, and fungal infections. PDT can selectively destroy these pathogens while leaving the healthy tissues intact [38,39].

PDT has been studied as a potential treatment for chronic wounds, such as diabetic foot ulcers. It can help to promote wound healing by stimulating the growth of new blood vessels and reducing inflammation.

3.4 Challenges faced by PDT as a treatment modality

Photodynamic therapy (PDT) has emerged as a promising treatment modality for various diseases. However, like all treatment options, PDT has limitations and challenges that must be taken into consideration. One of the primary limitations of PDT is its limited depth of tissue penetration. The treatment is best suited for treating superficial tumors or lesions close to the skin surface. In cases where the tumor is deeper, such as in certain types of cancer, PDT may not be effective [40,41].

Another challenge with PDT is its potential to induce photosensitivity. The photosensitizing agent used in PDT can make patients sensitive to light for several days after treatment. Patients must avoid sunlight or bright indoor lighting during this time to prevent skin damage. While PDT is selective for abnormal cells, it can also affect healthy cells in the treatment area, leading to inflammation and tissue damage. This lack of selectivity can be a challenge in treating certain diseases. Another challenge with PDT is the variable response seen in different patients. Some patients may respond well to PDT, while others may not. The efficacy of PDT may be influenced by a variety of factors, including the type of disease being treated, the stage of the disease, and the patient's overall health [28,42,43].

While PDT offers several advantages over conventional treatment modalities, it is not without limitations and challenges. Despite these limitations, PDT remains a promising treatment option for certain types of cancer and other diseases. Further research is needed in this area to refine the technique and improve its efficacy [36].

4 Immunotherapy

A rapidly developing area of medicine called immunotherapy uses the body's immune system to combat illnesses, particularly cancer. The immune system is a sophisticated system of cells, tissues, and organs that cooperate to defend the body against outside invaders like bacteria, viruses, and cancer cells [44]. Immunotherapy involves the use of various treatments to activate or enhance the immune system's ability to recognize the tumor and attack the cancer cells. Traditional cancer treatments such as chemotherapy and radiation therapy target rapidly dividing cells, which can also damage healthy cells in the body. Immunotherapy, on the other hand, specifically targets cancer cells, and the healthy cells are left unharmed. This targeted approach has made immunotherapy a promising treatment option for a variety of cancers, including leukemia, melanoma, and lung cancer, among others [45,46].

There are several types of immunotherapy, including monoclonal antibodies, immune checkpoint inhibitors, cancer vaccines, and adoptive cell transfer. Each of these treatments works in a different way to stimulate the immune system to fight cancer cells [44]. Fig. 3 shows the different forms of immunotherapy.

4.1 Principles of immunotherapy

The principles of immunotherapy involve manipulating the immune system to target and attack abnormal or diseased cells while sparing the healthy cells.

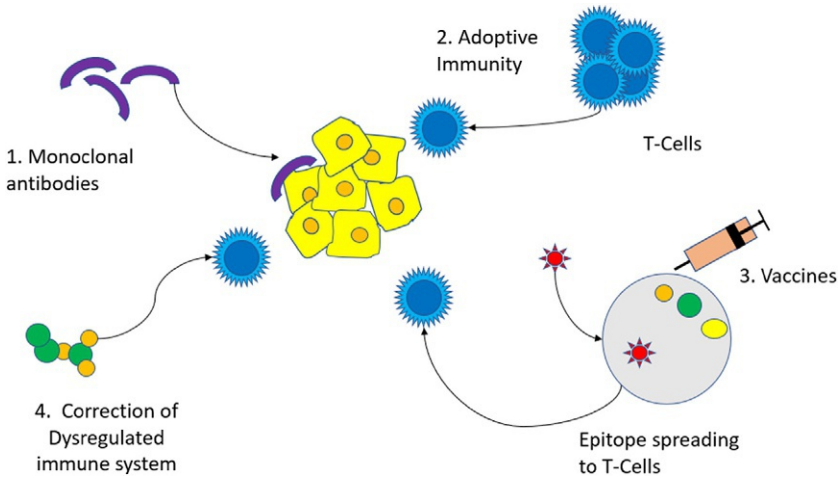

Fig. 3 Forms of immunotherapy.

There are several key principles of immunotherapy, which are explained in the following sections.

4.1.1 Recognition of the target

The first principle of immunotherapy is the recognition of the target, which is the abnormal or diseased cells that need to be eliminated. The immune system uses various mechanisms to recognize these targets, including surface markers, antigens, and other molecular structures that are unique to the target cells. Immunotherapies can be designed to target specific cells, such as cancer cells, by identifying these unique markers [47,48].

4.1.2 Activation of the immune response

Once the target has been recognized, the immune system needs to be activated to mount an effective response against the target. This involves activating immune cells, such as T cells and natural killer cells, which are responsible for attacking abnormal cells. Immunotherapies can be designed to activate these cells by stimulating the immune system with specific molecules, such as cytokines, or by using antibodies that bind to immune cells and activate them [49,50].

4.1.3 Modulation of immune response

The immune system is complex, and its response can be regulated by various factors, including inhibitory signals that prevent immune cells from attacking healthy tissues. The third principle of immunotherapy is the modulation of the immune response to ensure that it is appropriately targeted and effective. This can be achieved by blocking inhibitory signals, such as checkpoint inhibitors, or by enhancing the immune response using costimulatory signals [51,52].

4.1.4 Amplification of the immune response

Immunotherapies can be designed to amplify the immune response, making it more effective at eliminating the target cells. This can be achieved by boosting the number or activity of immune cells, such as T cells and natural killer cells, or by increasing the production of cytokines and other immune molecules [53,54].

4.1.5 Persistence of immune response

Finally, the fifth principle of immunotherapy is the persistence of the immune response, which is critical for long-term protection against the

target cells. This can be achieved by generating memory cells, which can quickly recognize and attack the target cells if they reappear. Immunotherapies can be designed to promote the generation of memory cells, ensuring that the immune response remains active even after treatment has ended [55,56].

4.2 Types of immunotherapy

There are several types of immunotherapy, each designed to target different aspects of the immune system and different diseases. In this response, we will discuss the most common types of immunotherapy:

- Monoclonal antibodies: Proteins called monoclonal antibodies are made to recognize and attach to particular chemicals found on the surface of cancer cells. They can either kill cancer cells directly as a result of this or trigger the immune system to fight them. Breast cancer, lymphoma, and leukemia are just a few of the cancers that can be treated with monoclonal antibodies [57].
- Checkpoint inhibitors: A kind of immunotherapy known as checkpoint inhibitors disables inhibitory signals that stop T cells from attacking cancerous cells. Cancer cells can make use of these inhibitory signals to elude the immune system, even though they are intended to stop the immune system from attacking healthy tissues. Checkpoint inhibitors can stimulate T lymphocytes and enable them to attack cancer cells by inhibiting these signals. Several forms of cancer, including melanoma, lung cancer, and bladder cancer, are treated with checkpoint inhibitors [58].
- CAR T-cell therapy: Immunotherapy, known as CAR T-cell treatment, includes genetically altering a patient's T cells so that they can detect and combat cancer cells. Chimeric antigen receptors (CARs), which can recognize particular chemicals on the surface of cancer cells, are engineered into T cells to do this. The T cells can identify and combat cancer cells if they are reintroduced into the patient. Certain forms of leukemia and lymphoma are treated with CAR T-cell therapy [59].
- Cytokines: Cytokines are proteins that are produced by the immune system and play a critical role in regulating the immune response. Some cytokines, such as interferons and interleukins, can be used in immunotherapy to stimulate the immune system to attack cancer cells. Cytokine therapy is used to treat certain types of cancer, including melanoma and kidney cancer [60].

- Vaccines: As a form of immunotherapy, vaccines work by encouraging the immune system to identify and target particular infections or cancerous cells. Cancer vaccines are designed to target specific molecules that are expressed on the surface of cancer cells, and by doing so, they can stimulate the immune system to attack cancer cells. Cancer vaccines are used to treat various types of cancer [61].
- Immune checkpoint modulators: One kind of immunotherapy called an immune checkpoint modulator can either activate or inhibit particular immunological checkpoints. These immune checkpoints are critical for maintaining the balance between immune activation and immune tolerance. By modulating these checkpoints, immune checkpoint modulators can either activate or inhibit the immune response to cancer cells. Immune checkpoint modulators are used to treat various types of cancer, including melanoma, lung cancer, and bladder cancer [54].

4.3 Advantages of immunotherapy over conventional therapies

Immunotherapy has various benefits over more traditional treatments such as chemotherapy and radiation therapy. Immunotherapy is a targeted therapy, which means that it directly targets cancer cells while sparing the healthy cells, and this is one of its main benefits. Contrastingly, conventional treatments such as chemotherapy and radiation therapy can harm both cancer cells and healthy cells. Immunotherapy can thereby lessen adverse effects and enhance patients' quality of life [62].

In order to increase the efficacy of other therapies like chemotherapy and radiation therapy, immunotherapy can also be employed in conjunction with them. For example, some immunotherapies can enhance the immune response to cancer cells, making them more susceptible to chemotherapy or radiation therapy. By combining different treatments, doctors can improve treatment outcomes and reduce the risk of cancer recurrence [63,64].

Furthermore, immunotherapy is a rapidly evolving field, with ongoing research focused on developing new and more effective immunotherapies. This means that patients have access to a wide range of treatment options, including targeted therapies and personalized medicine. As a result, patients can receive treatments that are tailored to their specific needs, maximizing the effectiveness of the treatment and minimizing side effects.

4.4 Clinical applications of immunotherapy in cancer and other diseases

Immunotherapy has revolutionized the treatment of cancer and other diseases, offering new and effective treatment options for patients. Here, we will discuss some of the clinical applications of immunotherapy in cancer and other diseases.

Immunotherapy has become a viable treatment option for a variety of cancers. Immune checkpoint inhibitors, which stop cancer cells from using certain signals to elude the immune system, are one of the most often utilized types of immunotherapy. Immune checkpoint inhibitors improve survival rates and have fewer adverse effects than conventional chemotherapy and radiation therapy because they enable the immune system to identify and target cancer cells [58,65].

CAR T-cell therapy is another type of immunotherapy used to treat cancer. In this procedure, T cells are drawn from a patient's blood, modified to target cancer cells, and then reinfused into the patient. In the treatment of blood cancers, including leukemia and lymphoma, CAR T-cell therapy has shown encouraging results, and current research is investigating its potential in the treatment of other cancers as well [59].

In addition to cancer, immunotherapy has also shown promise in the treatment of autoimmune disorders such as rheumatoid arthritis, multiple sclerosis, and lupus. By modulating the immune system, immunotherapy can reduce inflammation and slow the progression of these diseases. One type of immunotherapy used in the treatment of autoimmune disorders is monoclonal antibody therapy, which is designed to target specific molecules involved in the immune response [66,67].

Immunotherapy has also been used in the treatment of infectious diseases such as HIV and hepatitis B and C. In these cases, immunotherapy is used to boost the immune system's ability to recognize and attack the virus, leading to improved outcomes. For example, immune checkpoint inhibitors have been shown to improve outcomes in patients with advanced hepatocellular carcinoma, a type of liver cancer associated with hepatitis B and C [68].

Overall, the clinical applications of immunotherapy are rapidly expanding, offering new and effective treatment options for a wide range of diseases. Ongoing research is focused on developing new and innovative immunotherapies with the potential to transform the treatment of various diseases and improve patient outcomes.

4.5 Challenges faced by immunotherapy as a treatment modality

Although immunotherapy has shown promising results in the treatment of various diseases, it also faces several challenges that limit its effectiveness. Here, we will discuss some of the challenges faced by immunotherapy as a treatment modality.

One of the main challenges of immunotherapy is the development of resistance. Cancer cells, in particular, can evolve to evade the immune system, rendering immunotherapy ineffective. In some cases, cancer cells can even suppress the immune response, making it difficult for immunotherapy to be effective. This is a major challenge in the field of immunotherapy, and researchers are working to develop new strategies to overcome the resistance. One approach is to use combination therapy, where multiple drugs are used in conjunction to target multiple pathways and increase the chances of a response. Another approach is to use personalized medicine, where treatment is tailored to the specific genetic profile of the patient and their cancer [69–71].

Another challenge faced by immunotherapy is toxicity. Although immunotherapy can offer a more targeted approach to treatment compared to traditional chemotherapy and radiation therapy, it can still cause side effects. Some of the side effects can be severe, including inflammation of the lungs, liver, and other organs, or autoimmune reactions that attack healthy tissue. These side effects can limit the use of immunotherapy, particularly in patients with preexisting conditions [72,73].

Immunotherapy can also be expensive, which is a major barrier to access for many patients. Although the cost of immunotherapy has decreased in recent years, it remains prohibitively expensive for many patients, particularly in low-income countries or those without insurance. This highlights the need for more affordable treatment options to increase access to immunotherapy for all patients who may benefit from it [74].

Finally, immunotherapy is not effective for all patients. Some patients may not respond to immunotherapy, while others may experience only a partial response. This highlights the need for better patient selection and biomarkers to predict response to treatment. Ongoing research is focused on identifying biomarkers that can predict response to immunotherapy, allowing for more personalized treatment approaches [75].

5 Microneedle-mediated photodynamic/immunotherapy

Microneedle-mediated photodynamic/immunotherapy (MNPDI) is a cutting-edge technique that combines the benefits of PDT and immunotherapy to provide a targeted and effective treatment option for cancer and other diseases. This technique involves the use of microneedles to deliver photosensitizers and immunostimulatory agents directly into the tumor site, followed by the application of light to activate the photosensitizer and induce cell death. The use of microneedles provides several advantages over traditional delivery methods. Microneedles can penetrate the skin without causing significant damage, allowing for the targeted delivery of drugs directly into the tumor site. This approach minimizes the exposure of healthy tissues to the drugs and reduces the risk of systemic toxicity. In addition, microneedles can be used to create microchannels in the skin, which can increase the permeability of the skin and enhance drug delivery [1,76–78].

The technique of PDT involves the use of photosensitizers, which are molecules that can absorb light energy and transfer it to surrounding oxygen molecules, creating ROS that can induce cell death. When photosensitizers are delivered to the tumor site using microneedles, the targeted application of light can activate the photosensitizer and induce cell death specifically in the cancerous cells, leaving healthy cells unaffected [79].

Furthermore, PDT has been found to induce the release of DAMPs, which can activate the immune system and recruit immune cells to the site of the tumor. This immune response can enhance the effectiveness of the treatment by targeting not only the cancer cells but also the immune cells. To further enhance the immune response, immunostimulatory agents can be delivered to the tumor site using microneedles. Immunostimulatory agents are molecules that can activate the immune system and enhance the immune response. For example, immunostimulatory agents such as Toll-like receptor (TLR) agonists can activate immune cells and enhance their ability to recognize and attack cancer cells [80,81]. This technique has been shown to induce tumor cell death, enhance the immune response, and improve overall survival in animal models. The use of microneedles for drug delivery and immune stimulation offers a potentially effective and targeted approach to the treatment of cancer and other diseases, with minimal side effects and a reduced risk of systemic toxicity [82].

5.1 Rationale for combining microneedles, PDT, and immunotherapy

The rationale for combining microneedles, (PDT) and immunotherapy lies in the potential to enhance the effectiveness of cancer treatment by utilizing the unique advantages of each approach.

Microneedles offer a minimally invasive method for drug delivery that allows for targeted and precise delivery of therapeutic agents directly into the tumor site. Traditional delivery methods, such as intravenous infusion, can lead to systemic toxicity and limit the effectiveness of the treatment. By delivering the drug directly into the tumor site using microneedles, the amount of drug required can be reduced, which can lead to a reduction in side effects and an improvement in therapeutic efficacy [77,83].

PDT involves the use of photosensitizers, which are molecules that can absorb light energy and transfer it to the surrounding oxygen molecules, creating ROS that can induce cell death. This approach has been shown to be effective in the treatment of cancer, as it can selectively target cancer cells while minimizing damage to healthy tissues. In addition, PDT has been found to induce an immune response, which can further enhance the effectiveness of the treatment. Immunotherapy involves the use of the immune system to recognize and attack cancer cells. This approach has revolutionized cancer treatment, as it can provide long-lasting remission and potentially even cure the disease. However, not all patients respond to immunotherapy, and the response rate can vary depending on the type and stage of cancer [28,40,69,72].

Combining microneedles, PDT, and immunotherapy can provide a synergistic effect, as each approach can enhance the effectiveness of the other. Microneedles can be used to deliver photosensitizers and immunostimulatory agents directly into the tumor site, which can enhance the effectiveness of PDT and immunotherapy, respectively. The photosensitizers delivered using microneedles can selectively target cancer cells and induce cell death, while the immunostimulatory agents can activate the immune system and enhance the immune response against the cancer cells [16].

Furthermore, combining these approaches can potentially overcome the limitations of each individual approach. For example, PDT has been found to induce an immune response, but this response may not be strong enough to overcome the immunosuppressive environment in the tumor. By combining PDT with immunotherapy, the immune response can be further enhanced, potentially leading to better treatment outcomes [84,85].

Overall, combining microneedles, PDT, and immunotherapy offers a potentially effective and targeted approach to the treatment of cancer and other diseases with minimal side effects and a reduced risk of systemic toxicity. The synergistic effect of these approaches can enhance the effectiveness of each individual approach and potentially lead to better treatment outcomes.

5.2 Strategies for microneedle-mediated drug delivery in PDT/immunotherapy

Microneedle-mediated drug delivery is a promising strategy for enhancing the effectiveness of PDT and immunotherapy in the treatment of cancer and other diseases. There are several strategies for microneedle-mediated drug delivery in PDT/immunotherapy, including the use of various types of microneedles, the incorporation of different types of drugs, and the use of different delivery techniques.

One strategy is to use solid microneedles, which can be made of various materials, such as silicon, metal, or polymer. These microneedles can be coated with drugs, photosensitizers, or immunostimulatory agents, which can then be delivered directly to the tumor site. Solid microneedles can penetrate the skin and reach the desired depth within the tumor, where they can release the drug or photosensitizer to induce cell death or activate the immune system. Another strategy is to use dissolving microneedles, which are made of biodegradable materials that dissolve upon contact with the skin. These microneedles can be coated with drugs, photosensitizers, or immunostimulatory agents, which can then be released as the microneedles dissolve. Dissolving microneedles can provide a more sustained release of the drug or photosensitizer, which can potentially enhance the effectiveness of the treatment [86,87].

Incorporating multiple drugs or agents into a single microneedle array is another strategy for microneedle-mediated drug delivery. For example, a microneedle array can be coated with a photosensitizer and an immunostimulatory agent, which can then be delivered simultaneously to the tumor site. This approach can potentially enhance the effectiveness of both PDT and immunotherapy, as the two agents can work synergistically to induce cell death and activate the immune system.

In addition to the type of microneedle and the drugs or agents used, the delivery technique can also impact the effectiveness of microneedle-mediated drug delivery. For example, microneedles can be delivered using a mechanical applicator or a patch, which can impact the depth and distribution of the drug within the tumor site. Choosing the appropriate delivery technique can help ensure that the drug is delivered to the desired depth and

location within the tumor, which can potentially enhance the effectiveness of the treatment [88].

Overall, microneedle-mediated drug delivery offers a promising strategy for enhancing the effectiveness of PDT and immunotherapy in the treatment of cancer and other diseases. Choosing the appropriate type of microneedle, drug or agent, and delivery technique can impact the effectiveness of the treatment and improve treatment outcomes. By combining microneedles with PDT and immunotherapy, researchers and clinicians can potentially develop more effective and targeted treatments with fewer side effects and a reduced risk of systemic toxicity [14]. Fig. 4 shows a schematic of a possible mechanism by MN-assisted PDT in combination with immune checkpoint blockade for antitumor immune responses [89].

5.3 Preclinical and clinical studies of microneedle-mediated PDT/immunotherapy

Preclinical and clinical studies have shown promising results for microneedle-mediated photodynamic therapy and immunotherapy (MNPDI). These studies have demonstrated the potential for microneedle-mediated drug

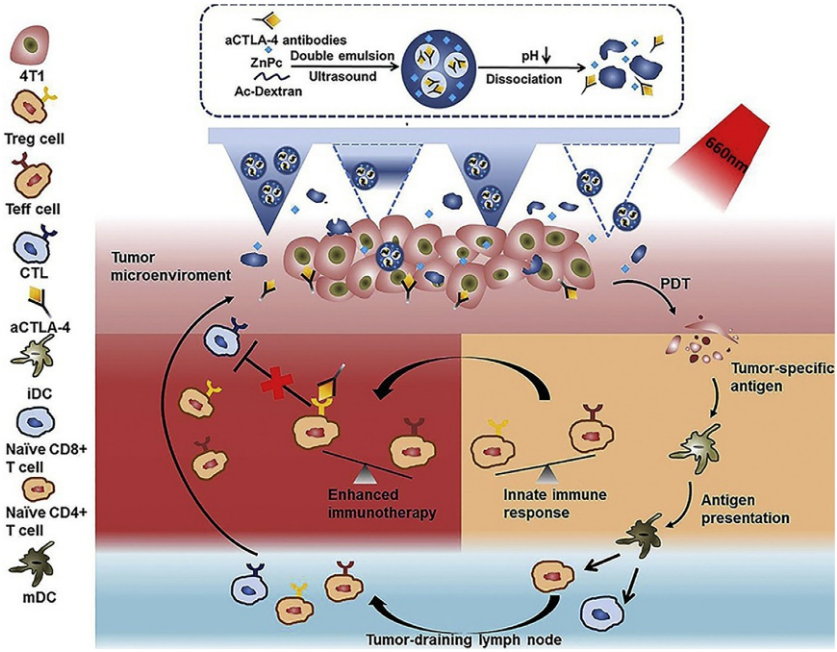

Fig. 4 Scheme of the MN-assisted codelivery system and possible mechanism of antitumor immune responses induced by MN-assisted PDT in combination with immune checkpoint blockade [89].

delivery to enhance the effectiveness of PDT and immunotherapy in the treatment of cancer and other diseases.

In preclinical studies, researchers have used solid and dissolving microneedles to deliver photosensitizers and immunostimulatory agents to tumor sites in animal models. These studies have shown that microneedle-mediated drug delivery can improve the effectiveness of PDT and immunotherapy compared to traditional delivery methods, such as intravenous injection or topical application. For example, one study showed that microneedle-mediated delivery of a photosensitizer and an immunostimulatory agent led to a significant reduction in tumor volume in a mouse model of breast cancer compared to traditional delivery methods [90,91].

In clinical studies, researchers have evaluated the safety and effectiveness of MNPDI in human patients with various types of cancer. One study evaluated the use of microneedles to deliver a photosensitizer for PDT in patients with basal cell carcinoma. The study found that microneedle-mediated PDT was safe and effective, with a high rate of complete response and low rates of adverse events [92].

Other clinical studies have evaluated the use of microneedles to deliver immunotherapy agents, such as checkpoint inhibitors or vaccines, to tumor sites in patients with melanoma or other types of cancer. These studies have shown promising results, with some patients experiencing complete responses or prolonged survival [93].

Overall, preclinical and clinical studies have shown that microneedle-mediated drug delivery can enhance the effectiveness of PDT and immunotherapy in the treatment of cancer and other diseases. These studies provide evidence for the potential of microneedle-mediated drug delivery as a novel and targeted approach for improving the effectiveness of cancer treatments [94]. However, further research is needed to optimize the use of MNPDI, including determining the optimal type of microneedle, drug or agent, and delivery technique for specific types of cancer and patient populations.

5.4 Advantages of microneedle-mediated PDT/immunotherapy

Microneedle-mediated photodynamic therapy and immunotherapy (MNPDI) offer several advantages over traditional treatment methods. This innovative technique involves the use of microneedles to deliver photosensitizers and immunotherapeutic agents directly to the target tissue, resulting in enhanced efficacy and reduced systemic toxicity. The microneedles create tiny channels in the skin, allowing for precise and controlled delivery of the therapeutic agents to the affected area. This targeted approach allows for

higher concentrations of the agents to be delivered to the tumor or lesion, which can result in a more potent therapeutic effect [89,95].

In addition to the precise delivery of therapeutic agents, MNPDI can also stimulate the immune system and promote a local immune response. The microneedles create microchannels in the skin, which can act as a signal to the immune system, alerting it to the presence of a foreign invader. This can lead to increased efficacy of the immunotherapy and a more robust anti-tumor immune response [96].

Another advantage of MNPDI is the potential for improved patient compliance. Traditional treatment methods, such as surgery or radiation therapy, can be painful and inconvenient for patients, which can lead to noncompliance with treatment protocols. However, the minimally invasive nature of MNPDI makes it less painful and more convenient for patients, which can improve adherence to treatment and lead to better outcomes [97].

5.5 Challenges faced by microneedle-mediated PDT/immunotherapy

While microneedle-mediated photodynamic therapy and immunotherapy (MNPDI) offer several advantages over traditional treatment methods, they also face several challenges. One of the main challenges is the development of microneedles that can effectively penetrate the skin and deliver therapeutic agents to the target tissue. The design and fabrication of microneedles can be complex, and the needles must be able to deliver the agents to the correct depth and at the appropriate concentration to ensure efficacy [89,96].

Another challenge is the potential for skin irritation and inflammation caused by the microneedles. The creation of microchannels in the skin can trigger an immune response, which can lead to discomfort and local swelling. This can be a particular concern for patients with sensitive skin or who are undergoing treatment in a highly visible area, such as the face [98].

The use of photosensitizers in PDT can also pose challenges. Photosensitizers can be toxic and can cause skin sensitivity to light, which can lead to adverse side effects such as phototoxicity. In addition, photosensitizers can have a short half-life, which can limit their effectiveness. Immunotherapy also faces challenges related to its effectiveness and safety. While immunotherapy has shown promise in treating cancer and other diseases, some patients do not respond to the treatment or may develop adverse reactions, such as immune-related adverse events. In addition, immunotherapy can be expensive, and the cost may limit its accessibility to some patients [41,99].

Overall, MNPDI has the potential to revolutionize the treatment of various diseases. However, overcoming the challenges associated with these techniques is essential for their widespread adoption and success. Ongoing research and development in this area are critical for improving the safety and efficacy of MNPDI and making them more accessible to patients.

6 Conclusion and future perspectives

Microneedles offer a promising approach to drug delivery due to their numerous advantages over conventional methods, such as painless administration, minimal invasiveness, and enhanced efficacy. PDT and immunotherapy are emerging treatment modalities for various diseases, including cancer, that offer several advantages over conventional therapies, such as lower toxicity, specificity, and potential for long-term remission. However, both PDT and immunotherapy also face challenges, such as limited penetration depth and immunogenicity, respectively [9].

The chapter discusses the rationale for combining microneedles, PDT, and immunotherapy, as well as the strategies and challenges associated with microneedle-mediated drug delivery in PDT/immunotherapy. Preclinical and clinical studies have shown promising results for microneedle-mediated PDT/immunotherapy, with several advantages over conventional approaches [83].

Overall, the combination of microneedles, PDT, and immunotherapy represents a promising approach for the treatment of various diseases. However, further research is needed to overcome the challenges and optimize the efficacy and safety of this approach. Future studies should also focus on developing novel microneedle designs and optimizing drug formulations for improved drug delivery [100].

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CHAPTER 16

Microneedle-mediated gene delivery: A promising approach for diverse disease treatment

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1 Introduction

Gene delivery, also known as gene transfer or gene therapy, refers to the transfer of genetic material into a cell or an organism to treat or prevent disease. The technique of gene delivery has revolutionized the field of biomedicine and has immense potential to cure previously incurable diseases. Gene delivery can be used to deliver new or replacement genes, as well as to regulate the expression of endogenous genes [1–3]. The earliest attempts at gene delivery were made in the 1970s, when researchers attempted to introduce exogenous DNA into mammalian cells using a variety of techniques, including electroporation, lipofection, and calcium phosphate transfection. These early methods were often inefficient and had limited success due to issues such as low transfection efficiency, low expression levels, and the inability to target specific tissues or cells [4–6]. Over the years, developments in biotechnology and molecular biology have led to the development of new and more efficient methods of gene delivery [7]. The introduction of viral vectors, such as retroviruses, lentiviruses, and adenoviruses, as well as nonviral methods, such as electroporation, ultrasound, and gene guns, has greatly improved the efficiency and specificity of gene delivery [6]. Viral vectors are currently the most commonly used method of gene delivery due to their ability to efficiently transduce a variety of cell types and tissues. These vectors are engineered to carry therapeutic genes and are typically derived from viruses that have been modified to eliminate their pathogenicity. The use of viral vectors for gene delivery has led to successful clinical trials for a range of genetic diseases, including hemophilia, cystic fibrosis, and muscular dystrophy [8,9]. Nonviral methods of gene delivery,

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on the other hand, have the advantage of being safer and easier to manufacture than viral vectors [10]. These methods include electroporation, which involves the use of electric fields to introduce DNA into cells, and gene guns, which use high-pressure gas to deliver DNA-coated particles directly into cells. While these methods have lower transfection efficiencies than viral vectors, they are effective for delivering therapeutic genes to certain tissues and organs, such as the skin and the eye [11].

The development of gene editing technologies, such as CRISPR/Cas9, has further expanded the potential of gene delivery [12]. These technologies allow for precise manipulation of the genome, enabling researchers to correct disease-causing mutations, delete or insert genes, and regulate gene expression. Gene editing technologies have already been used in clinical trials for a variety of genetic disorders, including beta thalassemia and sickle cell anemia [13,14]. Despite the promise of gene delivery for the treatment of genetic diseases, significant challenges must be overcome. One major challenge is the potential for immune responses to the therapeutic gene or the vector used for delivery. This can destroy the transduced cells and the loss of therapeutic effect [15]. Another challenge is the need for more efficient and specific delivery methods that can target specific tissues and cells without causing off-target effects [16]. Overall, gene delivery has revolutionized the field of biomedicine and has immense potential for the treatment of genetic diseases. The continued development of new and more efficient methods of gene delivery and the advancement of gene editing technologies will undoubtedly lead to further breakthroughs in the field. However, careful consideration must be given to gene therapy's safety and ethical implications, and research must continue to address the challenges associated with gene delivery [17,18].

Currently, the primary obstacle is to develop a secure and effective mechanism for genetic materials to accurately reach the intended cells. One relatively novel approach to gene delivery involves utilizing microneedles (MNs), which have undergone extensive research for various molecule types and indications. MNs can traverse the stratum corneum (SC), the primary barrier for drug delivery through the skin, with minimal discomfort and simplified administration [19]. MNs have emerged as a promising technology for delivering gene therapies. These tiny needles, typically smaller than a millimeter in length, can penetrate the skin's outermost layer to deliver therapeutic agents, including RNA and DNA, directly into the cells beneath [20]. This method offers several advantages over traditional gene delivery techniques, such as injections and electroporation. MNs can be

painless and minimally invasive, making them ideal for noninvasive gene delivery in patients who might be hesitant to undergo more invasive procedures. Additionally, they offer the potential for improved targeting and localized delivery, reducing the risk of off-target effects and increasing the effectiveness of the therapy [21].

2 The disadvantage of existing methods/existing problems of gene delivery

2.1 Viral vectors

Viral vectors are the most commonly used method of gene delivery due to their ability to efficiently transfer genes into a variety of cell types and tissues. Viruses are naturally evolved to deliver their genetic material into host cells, making them effective vectors for gene therapy [22]. Viral vectors are developed by modifying the viral genome to remove any disease-causing genes and replacing them with therapeutic genes. The viral vector is then used to infect target cells, delivering the therapeutic gene into the cells [23]. There are several types of viral vectors commonly used in gene delivery, including lentiviral vectors, retroviral vectors, adenoviral vectors, and adeno-associated viral vectors (AAVs) [24,25]. Overall, the choice of viral vector depends on the specific application and the characteristics of the target cells. Despite their potential benefits, viral vectors have limitations, including the risk of insertional mutagenesis and the potential for immune reactions [26]. Therefore, ongoing research is focused on improving the efficacy and safety of gene delivery using viral vectors. The limitation of viral vectors in gene delivery is listed in Table 1.

2.2 Nonviral vectors

Nonviral vectors are an alternative to viral vectors for gene delivery. These vectors are typically noninfectious and nonimmunogenic, making them a safer and more practical option for gene therapy [39]. Some common nonviral vectors used for gene delivery include liposomes, nanoparticles, and polymers [40,41]. Liposomes are spherical lipid-based structures that can be used to encapsulate DNA or RNA molecules. These liposomes can fuse with the cell membrane, allowing the RNA or DNA to enter the cell [42]. Nanoparticles and polymers are other nonviral vectors that can be used to deliver genetic material into cells. These vectors can be designed to target specific cells or tissues and can also protect the DNA or RNA from degradation in the body [43].

Table 1 Limitations of viral and nonviral vectors in gene delivery.

Sr. no.	Limitations	Details	Refs.
Viral vectors			
1.	Immune responses	Viral vectors can trigger an immune response, leading to inflammation and the destruction of the infected cells. This immune response can also affect the efficacy of subsequent treatments using the same vector	[27,28]
2.	Insertional mutagenesis	Viral vectors can integrate their DNA into the host genome, which can lead to the activation or inactivation of genes, causing unintended consequences	[29]
3.	Limited cargo capacity	Viral vectors have a limited capacity to carry the desired genetic material, which can limit their use in gene therapy	[30]
4.	Safety concerns	Viral vectors can cause unintended side effects, such as toxicity and tissue damage. Some viral vectors may also replicate and spread beyond the targeted area, increasing the risk of adverse events	[29,31]
5.	Manufacturing challenges	Viral vectors can be difficult to manufacture and can be time-consuming and expensive. Additionally, the quality of viral vectors can be difficult to control, leading to variability in their effectiveness	[32]
Nonviral vectors			
1.	Limited gene delivery	Nonviral vectors have a lower gene delivery efficiency compared to viral vectors. They can only carry small amounts of genetic material, which limits the number of cells that can be targeted	[4,33]
2.	Low transfection efficiency	Nonviral vectors have low transfection efficiency, which is the ability to introduce genes into target cells. This limits their therapeutic potential	[34]

Table 1 Limitations of viral and nonviral vectors in gene delivery—cont'd

Sr. no.	Limitations	Details	Refs.
3.	Short-term expression	Nonviral vectors do not integrate into the host genome, and as a result, the expression of the gene is short lived. The gene expression may last for only a few days or weeks	[35]
4.	Toxicity	Nonviral vectors can be toxic to cells, which can lead to cell death and inflammation. The toxicity can also affect the immune system, which may cause adverse reactions	[36]
5.	Immunogenicity	Nonviral vectors can trigger an immune response, which can cause the body to reject gene therapy. This can limit the efficacy of gene therapy	[37]
6.	Limited tissue specificity	Nonviral vectors lack tissue specificity, which means they cannot target specific tissues or organs. This can limit the efficacy of gene therapy and cause off-target effects	[38]

Nonviral vectors have several advantages over viral vectors, including lower toxicity, reduced immune response, and easier manufacturing and storage. However, their efficiency of gene delivery is usually lower than that of viral vectors. Therefore, the development of efficient nonviral vectors with high transfection efficiency and minimal toxicity remains an important area of research for gene therapy [44]. The limitation of nonviral vectors in gene delivery is listed in Table 1.

2.3 Physical methods

Physical methods of gene delivery involve using physical forces to introduce DNA or RNA into cells, without the need for any chemical or biological agents [45]. Some common physical methods of gene delivery include electroporation, microinjection, particle bombardment, and ultrasound. In electroporation, a high-voltage electrical pulse is applied to the target cells to create temporary pores in the cell membrane, allowing RNA or DNA to enter the cell [46]. In microinjection, a fine needle injects DNA or RNA directly into the target cell's nucleus [47]. In particle bombardment, DNA or RNA is coated onto microscopic metal particles, which are

then accelerated into the target cells using a particle gun [48]. In ultrasound-mediated gene delivery method, ultrasound waves are used to create transient pores in the cell membrane, allowing RNA or DNA to enter the cell [49].

Physical methods of gene delivery have several advantages over chemical or biological methods, including higher efficiency and specificity, reduced toxicity, and less immunogenicity [50]. However, these methods also have some limitations, such as the need for specialized equipment and expertise and the potential for cell damage and loss of viability.

3 MNs for gene delivery

MNs are a rapidly developing technology for drug delivery, including gene delivery. They are small, minimally invasive devices that can be used to deliver genes and other therapeutic molecules to specific tissues or cells. MNs are typically less than 1 mm in length and are made from biocompatible materials such as stainless steel, silicon, or biodegradable polymers [51]. Traditional gene delivery methods, such as viral vectors, have limitations, including safety concerns and limited cargo capacity. MNs offer several advantages over these methods, including ease of use, improved safety, and increased efficiency. One of the main advantages of MNs for gene delivery is their ability to target specific tissues or cells. MNs can be designed to penetrate specific layers of skin or other tissues, allowing genes to be delivered directly to the target cells. This targeted approach reduces the risk of off-target effects and increases the efficiency of gene delivery [52].

MNs also offer improved safety compared to traditional gene delivery methods. Because they are minimally invasive, MNs do not require surgical procedures or injections, reducing the risk of infection or other complications. In addition, MNs can be designed to dissolve after use, further reducing the risk of adverse effects. Another advantage of MNs for gene delivery is their ability to increase the efficiency of gene delivery [53,54]. MNs can be designed to create small channels in the target tissue, allowing genes to diffuse more easily into the cells. In addition, MNs can be coated with materials that enhance gene delivery, such as lipids or polymers [55]. Several different types of MNs can be used for gene delivery. Solid MNs are the simplest type, consisting of a single, solid needle [56]. Hollow MNs have a small channel that allows genes to be delivered directly into the target tissue [57]. Dissolving MNs are made from biodegradable materials and dissolve after insertion, releasing the genes into the target tissue [58]. MNs offer several

advantages for gene delivery, including targeted delivery, improved safety, and increased efficiency. While the technology is still in development, MNs show great promise as a tool for delivering therapeutic genes to specific tissues and cells and may have the potential to revolutionize the field of gene therapy in the future.

3.1 Solid microneedles

Solid MNs are tiny needles that penetrate the skin and can be used for various applications, including drug delivery and gene delivery [59]. Solid MNs for gene delivery are made of biocompatible materials such as silicon, metal, or polymers. These MNs are typically 100–1000 μm in length and 10–100 μm in diameter, with a sharp tip that can penetrate the skin without causing significant damage [60]. The delivery of genes using solid MNs is a relatively new technique that offers several advantages over traditional gene delivery methods. Solid MNs can deliver genes directly to the skin, which is a rich source of immune cells that can uptake and process foreign DNA. This means that gene delivery using solid MNs can induce a potent immune response and a more targeted therapeutic effect [61].

Solid MNs also offer a painless and noninvasive method of gene delivery. Unlike traditional gene delivery methods, which often require injection or surgery, solid MNs can be applied to the skin without causing any pain or discomfort. Typically, when using solid MNs, a two-step process is required. Initially, they pierce the SC to create temporary microchannels, followed by the application of a drug solution, gel, cream, or ointment in the form of a patch onto the skin surface [62,63]. Overall, solid MNs for gene delivery represent a promising new approach to gene therapy that offers several advantages over traditional methods. While more research is needed to fully understand the potential of this technique, early studies have shown promising results and suggest that solid MNs could be a valuable tool in the fight against genetic disease. The schematic representation of gene delivery using solid MNs is shown in Fig. 1.

3.2 Coated microneedles

Coated MNs are a type of drug delivery system that involves the use of micron-sized needles coated with a thin layer of biocompatible material. These MNs are typically made from materials such as stainless steel or silicon and are designed to penetrate the outer layer of the skin to deliver drugs or

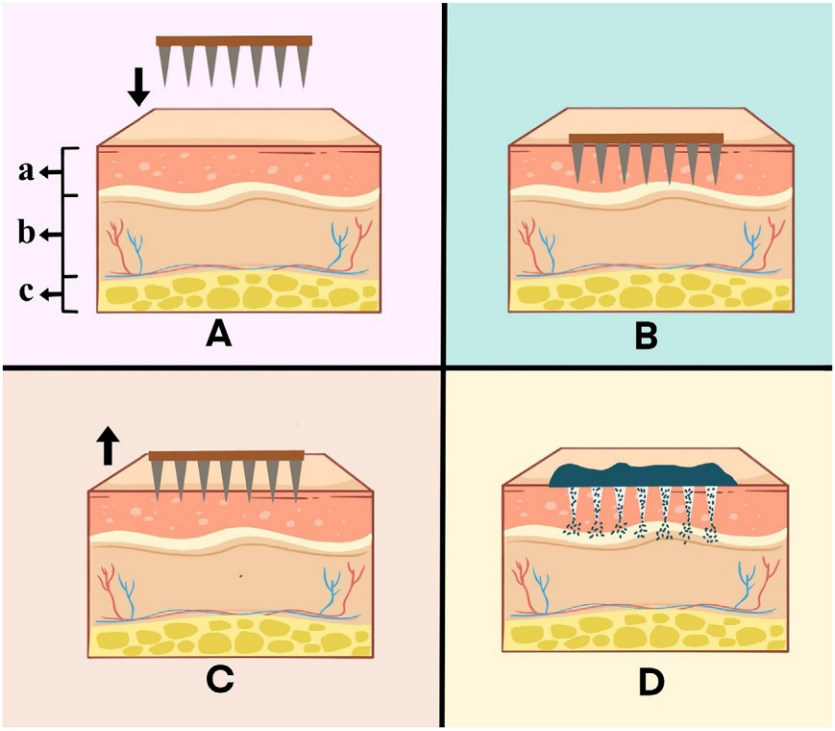

Fig. 1 The schematic representation of gene delivery using solid microneedles. (a) Epidermis, (b) dermis, and (c) hypodermis.

genes directly into the underlying tissue [64,65]. In the field of gene therapy, coated MNs have shown great promise as a novel and efficient method for delivering genes into cells. Gene delivery via MNs involves the use of plasmid DNA or viral vectors, which are loaded onto the surface of the MNs before application. There are many benefits of coated MNs for gene delivery. One of the main advantages is that the MNs can target specific areas of the body, allowing for localized gene expression. They can also be designed to penetrate different depths of the skin, making them suitable for a range of applications. Coated MNs are minimally invasive, reducing the risk of infection and other complications [66]. The process of using coated MNs for gene delivery involves several steps. First, the gene of interest is loaded onto the surface of the MNs. Next, the coated MNs are applied to the skin and penetrate the outer layer. Once the MNs have penetrated the skin, the gene of interest is released into the surrounding tissue [67,68]. The schematic representation of gene delivery using coated MNs is shown in Fig. 2.

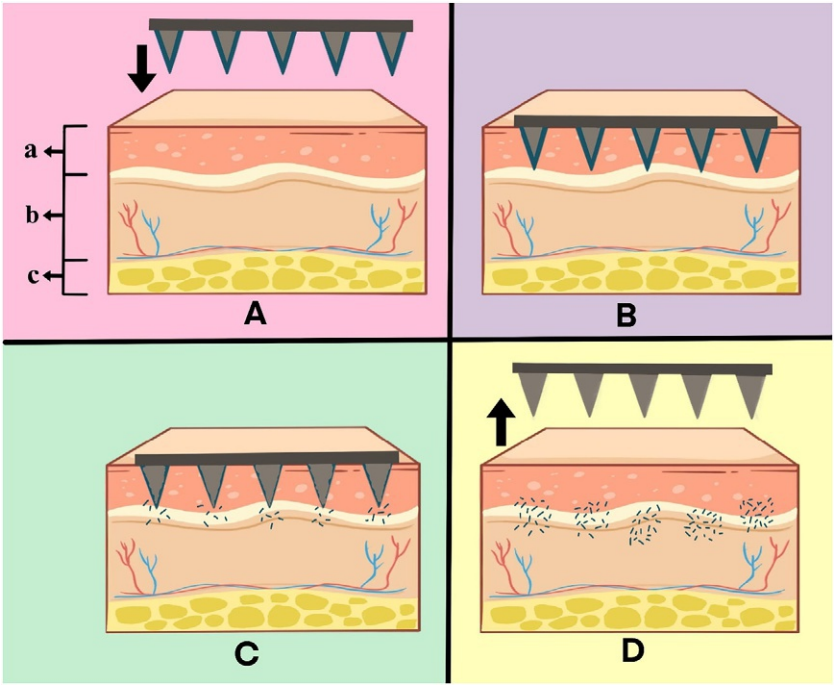

Fig. 2 The schematic representation of gene delivery using coated microneedles. (a) Epidermis, (b) dermis, (c) hypodermis.

Coated MNs have been used successfully in several preclinical and clinical trials for gene therapy applications, including the treatment of skin diseases, cancer, and genetic disorders. These trials have demonstrated that coated MNs are effective, safe, and well-tolerated by patients [66,68]. Overall, coated MNs represent a promising new approach to gene delivery that has the potential to revolutionize the field of gene therapy. With continued research and development, these devices could lead to more efficient, targeted, and personalized treatments for a range of diseases and disorders.

3.3 Hollow microneedles

Hollow MNs have been explored as a potential tool for gene delivery. They offer a minimally invasive and painless way to deliver genetic material directly into cells, bypassing the need for viral vectors or other invasive delivery methods. They can be used to deliver gene therapy agents such as plasmid DNA, siRNA, and viral vectors directly into the skin or underlying tissues [69]. The idea is that MNs can be used to create tiny pores in the

cell membrane, through which genetic material can be delivered. These MNs are typically made from materials such as metal, silicon, or polymer and can range in size from a few microns to a few hundred microns [70]. Hollow MNs deliver viral vectors, such as adenovirus or lentivirus, directly into cells. The viral vectors can then deliver the genetic material to the nucleus of the cell, where it can potentially alter gene expression. However, there are still several challenges associated with this type of MN in gene delivery, such as the need to optimize the microneedle design and the development of safe and effective gene delivery agents [71,72]. The schematic representation of gene delivery using hollow MNs is shown in Fig. 3.

While there is still much research to be done in this area, hollow MNs have shown promise as a potential tool for gene delivery. They offer a minimally invasive and potentially painless way to deliver genetic material directly into cells, which could have a wide range of applications in both clinical settings and research. Nonetheless, the potential benefits of hollow MNs in gene therapy delivery make them a promising avenue for further research and development.

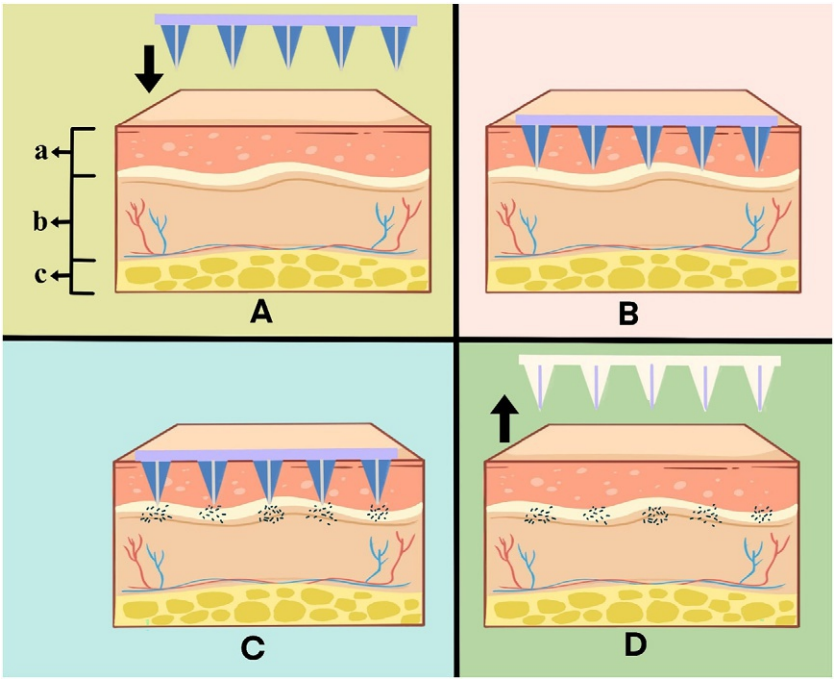

Fig. 3 The schematic representation of gene delivery using hollow microneedles. (a) Epidermis, (b) dermis, (c) hypodermis.

3.4 Dissolving microneedles

Dissolving MNs are tiny needles that dissolve in the skin and deliver drugs or other therapeutic agents. Combining dissolving MNs with gene delivery has been a promising approach for localized and targeted gene therapy. Dissolving MNs in gene delivery involves embedding plasmid DNA, small interfering RNA (siRNA), or other genetic material in a dissolvable polymer matrix. The MNs are then applied to the skin, where they puncture the outer layer and dissolve, releasing the genetic material into the skin [73]. One of the advantages of this approach is that it allows for a painless and noninvasive method of gene delivery. Additionally, the MNs provide a controlled release of the genetic material, which can be optimized to achieve the desired therapeutic effect. The dissolution of the MNs is a critical step for effective gene delivery. The rate of dissolution should be tailored to ensure that the genetic material is released in a controlled and sustained manner to allow for efficient transfection of the target cells. Additionally, the dissolution should occur without causing any significant damage or inflammation to the skin [74]. The dissolvable polymer matrix used for the MNs can be tailored to achieve specific release kinetics, depending on the gene therapy application. Several factors influence the dissolution rate of DMNs, including the size, shape, and material of the MNs, as well as the environmental conditions during the dissolution process. For example, a rapid release of the genetic material may be desired for vaccines, while for long-term gene therapy, a slow release may be more appropriate [75,76].

Overall, dissolving MNs in gene delivery offers a promising and innovative approach for localized and targeted gene therapy. It is still in the early stages of development but has shown potential for a variety of applications, including vaccination, gene editing, and cancer therapy. The schematic representation of gene delivery using dissolving MNs is shown in Fig. 4.

4 MNs in combination with other physical technologies

Physical methods of gene delivery involve using physical forces to introduce DNA or RNA into cells, without the need for any chemical or biological agents. When it comes to gene therapy, MNs have shown promise in enhancing the efficiency of gene delivery, particularly when used in combination with other physical technologies. Some of the physical technologies that can be used in combination with MNs for gene delivery include electroporation, ultrasound, jet injection, and laser ablation. However, these methods also have some limitations, such as the need for specialized

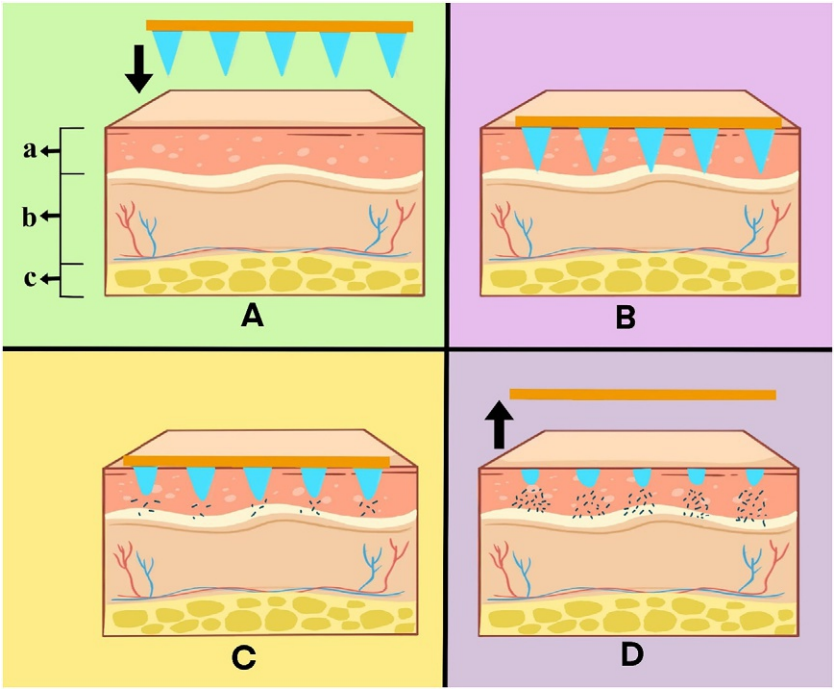

Fig. 4 The schematic representation of gene delivery using dissolving microneedles. (a) Epidermis, (b) dermis, (c) hypodermis.

equipment and expertise and the potential for cell damage and loss of viability. To address these issues, a suggested approach is to integrate these technologies with an MN platform. MNs are small, minimally invasive devices that can be used to create micropores in the skin, which can be used to deliver drugs or other therapeutic agents directly into the body. The schematic representation of physical methods for transdermal delivery is shown in Fig. 5.

Hooper et al. completed the first vaccination trial in an animal model employing MN-mediated electroporation for immunization. In the study, the two-part Easy Vax DNA vaccine delivery method was employed. An electrically conductive stainless steel MN array was coated with therapeutic DNA and a device created particularly to implant the array into the skin and provide electrical pulses that will improve DNA transport into cells. According to the study, plasmid DNA-coated MN arrays can be used to administer the 4pox DNA vaccine efficiently. When mice were given the 4pox DNA vaccine, they produced strong immune responses to the four immunogens

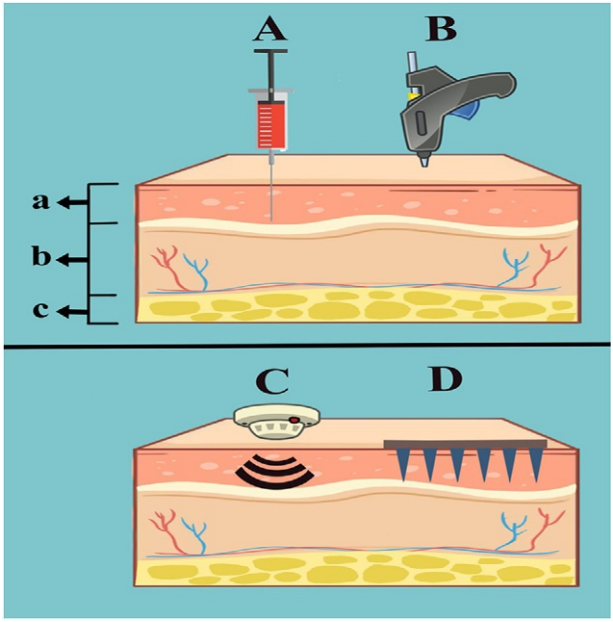

Fig. 5 The schematic representation of physical methods for transdermal gene delivery. (a) Epidermis, (b) dermis, (c) hypodermis, (A) intradermal injection, (B) gene gun, (C) ultrasound method, (D) microneedles.

of interest and neutralizing antibody titers that were higher than those produced by the standard live virus vaccination given via scarification. A lethal intranasal vaccinia virus challenge was also completely avoided by fully vaccinated animals [77].

Wei et al. reported a recent development in the use of MNs in combination with electroporation. Their research examined the utilization of low-voltage electroporation with a unique flexible MN array electrode (MNAE) chip to reduce the local tissue damage caused by electroporation and maximize cellular absorption of plasmid DNA delivered into healthy muscle tissue. According to the study, MNAE with high tissue surface coverage and effective low-voltage electroporation is available. Tissue damage and high-efficiency nucleic acid delivery were both validated during MNAE chip testing on mice. In vivo delivery of nucleic acid for DNA vaccination or gene therapy via targeted, minimally invasive electroporation is made possible by the MNAE chip [78].

In short, hollow conductive MNs were used to deliver DNA cargo intradermally and to stimulate the skin with electrical pulses. This method was

successful in eliciting an immune response to the model antigen ovalbumin by injecting proteins into the skin. Nevertheless, due to the dispersion of the electric field in the skin or the modest dosage of DNA supplied, numerical studies showed that the setup had certain limits for DNA electrotransfer. More research is required to get over these issues and evaluate this system's potential for enhancing the electrotransfer of DNA into the various skin layers [79].

Overall, the use of MNs in combination with other physical technologies holds great promise for enhancing the efficiency of gene delivery and making gene therapy more effective. However, more research is needed to fully understand the potential benefits and limitations of these approaches and to optimize their use for specific applications.

5 Application of microneedles in gene delivery

Gene therapy or replacing genes can be applied with nucleic acid to treat various genetic skin conditions, wound healing, and cutaneous malignancies. In recent years, DNA delivery through the transdermal route has had advantages such as large surface area, avoiding first-pass metabolism, and localized delivery. MNs have many potential applications in gene delivery, including drug delivery, vaccination, gene editing, and tissue engineering. The development of microneedle-based gene delivery systems is a rapidly evolving field that holds great promise for the treatment of many diseases. For various genetic diseases, genetic immunization, cancer, and wound healing, nucleic acids like plasmid DNA are delivered through MNs to the skin to improve the disease condition [51].

5.1 Cancer

MNs have been explored for gene delivery for cancer therapy. Gene therapy is a promising approach to cancer treatment that involves introducing therapeutic genes into cancer cells to inhibit their growth or induce their death. Recently, a study on the use of coated MNs for the delivery of siRNA nano complexes targeting BRAF for antimelanoma treatment have been reported. The researchers coated MNs with a cationic polymer and loaded them with octa arginine-conjugated BRAF siRNA nano complexes. They then delivered the coated MNs into the skin of Balb/c nude mice with melanoma and found that this method effectively delivered the siRNA nano complexes to the tumors, resulting in significant inhibition of tumor growth. The MN-mediated delivery also showed lower toxicity compared to

traditional intravenous delivery. Overall, this study suggests that coated MNs could be a promising approach for the targeted delivery of siRNA nanocomplexes for antimelanoma treatment [80]. The researchers used an industrialized ultrasonic spraying technology to coat polycaprolactone MNs with a polycation vector containing the plasmid DNA. They then applied the patch to subcutaneous tumors, and in vivo studies found that it effectively delivered the plasmid DNA to the tumors, resulting in improved antitumor efficacy. The MN patch rapidly dissolved in the skin, leaving no residue or damage and did not cause any adverse effects [81].

In skin cancer, the hyperactivity of STAT3 causes angiogenesis and melanoma. To target STAT3, the siRNA-based gene delivery loaded in MNs is used to treat skin cancer. As a result, using siRNA to reduce the risk of cancer by downregulating STAT3 activity and fabricating DMNs with the mixture of siRNA and polyethylenimine as a carrier was used to improve the efficiency of cellular uptake of siRNA. Skin insertion studies revealed that the MNs penetrated the skin by applying a force of 20 N, and in 1–5 min, the MNs were dissolved in the skin. Furthermore, in vitro studies on B16F10 cells and in vivo studies revealed that the combination of STAT3 siRNA and polyethylenimine increases the transfection efficiency by cell uptake [82]. The researchers designed an MN patch that contained a p53DNA and IR820 for photothermal therapy. They then applied the patch to subcutaneous tumors in mice and found that it effectively delivered the p53 DNA and IR820 to the tumors, resulting in synergistic gene and photothermal therapy that significantly inhibited tumor growth. The MN patch rapidly dissolved in the skin, leaving no residue or damage and did not cause any adverse effects [83].

5.2 Vaccine delivery

Vaccines are a crucial tool in preventing infectious diseases, but traditional vaccines often require injection with a needle and syringe, which can be painful and may cause anxiety for some individuals. Microneedle-based vaccines offer a potential solution to these issues, as they are painless and can be self-administered. MNs can also improve the immune response to vaccines, as they can directly target the immune cells in the skin. A new method for delivering plasmid DNA to the skin using Dermaroller MN (type of solid MNs) rollers coated with cationic poly (lactic-*co*-glycolic acid) (PLGA) nanoparticles. This technique can be used for transcutaneous immunization, which involves delivering vaccines directly to the skin to elicit an immune

response. The researchers demonstrated that the MNs coated with cationic PLGA nanoparticles were able to effectively deliver plasmid DNA to the skin, resulting in successful immunization against the influenza virus in mice. This method has the potential to be a more efficient and targeted way to deliver DNA vaccines for immunization purposes [84]. A study on the use of hollow MNs for the delivery of ovalbumin (OVA)-polymeric nanoparticles for intradermal vaccinations was conducted. They found that the MN-mediated delivery significantly enhanced both the cellular and humoral immune responses to the vaccine compared to intramuscular injection of the same vaccine [85].

In a study, the steel MNs were fabricated to deliver siRNA into the skin, as siRNA-based gene therapies are used to manage the disease conditions caused by abnormal gene expressions like malignant skin conditions or hyperproliferation. Chong et al. developed a steel MN coated with siRNA and conducted in vitro studies on HaCaT keratinocyte cells, in which the siRNA plays an essential role in reducing the level of Lamin A/C mRNA and protein. The commercial lipid-based transfection reagent was precomplexed with siRNA. Postcomplexation the lipid-based transfection reagent became less functional as microneedle coating with siRNA particularly targets CD44 and was modified as self-administrable, minimally invasive, and patient friendly application system to deliver nucleic acids into the skin [86]. The use of dissolving MNs for the delivery of a DNA vaccine against tuberculosis was carried out by Yan et al. The researchers designed MNs containing a plasmid DNA encoding the Ag85B antigen of *Mycobacterium tuberculosis* and delivered them into the skin of mice. They found that the MN-mediated delivery significantly enhanced both the cellular and humoral immune responses to the vaccine compared to intramuscular injection of the same vaccine. Overall, this study suggests that dissolving MNs could be a promising approach for enhancing the immunogenicity of DNA vaccines against tuberculosis [87]. The researchers loaded the MNs with a plasmid DNA encoding the hepatitis B virus (HBV) surface antigen and adjuvanted them with cationic liposomes and CpG ODN, which are known to enhance immune responses. They then delivered the MNs into the skin of mice and found that this method induced higher levels of both cellular and humoral immunity compared to intramuscular injection of the same vaccine without adjuvants. The study suggests that dissolving MN arrays adjuvanted with cationic liposomes, and CpG ODN could be a promising approach for DNA-based vaccination against HBV [88].

5.3 Parkinson's disease

Parkinson's disease is a neurodegenerative disorder that affects millions of people worldwide. The agonists and neurotransmitters were used to reduce the symptoms of Parkinson's disease. As a result, the drugs used to reduce the symptoms are ineffective because of a lack of continuous monitoring of the patients. Hence, electrochemical sensor-based MNs were used to monitor the apomorphine, a drug for managing Parkinson's disease. This apomorphine sensor is based on carbon paste loaded in hollow MNs, which are transduced using chronoamperometry and square wave voltammetry. The tertiary amines and oxidation peaks of apomorphine in the interstitial fluid are detected by the MN sensors. By using square wave voltammetry and chronoamperometry, the detection limit of the MN sensor is 0.6 and 0.75 M in an artificial interstitial fluid medium [89].

5.4 Myocardial diseases

In cardiovascular diseases, the drugs delivered through intramyocardial injections to the myocardium have drawbacks like limited retention of agents and restriction in needle-based injections. Hence, the adeno-associated virus-coated phase transition MNs were used, and the homogenous distribution of the adeno-associated virus was achieved. The delivery of adeno-associated virus-luciferase was detected by bioluminescence imaging. The MNs loaded with the adeno-associated virus vascular endothelial growth factor helps in functional angiogenesis. It improves heart function by enhancing the expression of vascular endothelial growth factor and activation of the AKT signaling pathway. As a result, MNs are used as an emerging tool for treating myocardial diseases [90].

6 Challenges/limitations of microneedles in gene delivery

MNs have gained significant attention as a promising device for gene delivery due to their potential to overcome the limitations of traditional needle-based injections. MNs are microscopic needles that can penetrate the skin without causing any significant damage or pain. However, while MNs offer several benefits, there are some limitations associated with their use in gene delivery. The limitations of MNs in gene delivery are discussed in Table 2.

Table 2 Challenges/limitations of microneedles in gene delivery.

Sl. no.	Challenges	Details	Refs.
Challenges/limitations of microneedles in gene delivery			
1.	Limited depth of penetration	One of the significant disadvantages of MNs is their limited depth of penetration. MNs can penetrate only up to a depth of 1 mm, which may not be sufficient for delivering genes to deep tissues, such as the muscle, bone, or liver. This limitation is due to the size of MNs, which is generally between 100 and 1000 μm. Therefore, MN-based gene delivery is restricted to the delivery of genes to the skin and shallow subcutaneous tissues	[91,92]
2.	Low gene delivery efficiency	MNs have lower gene delivery efficiency compared to traditional needle-based injections. While MNs can penetrate the skin without causing any significant damage, they do not have the same level of force as conventional needles, which can limit the amount of genetic material delivered to the target cells. Moreover, MNs may not be able to deliver the same quantity of genetic material as conventional needles, which can further reduce their gene delivery efficiency	[93,94]
3.	Difficult to target specific cells	Another disadvantage of MNs in gene delivery is the difficulty in targeting specific cells. MNs are designed to deliver genes to the skin or shallow subcutaneous tissues, which contain a variety of different cell types. Therefore, it is challenging to target specific cells with MNs, which can limit their therapeutic potential in some applications. Additionally, the use of MNs for gene delivery requires careful consideration of the desired target cells and the appropriate gene delivery vehicle	[20,59]

Table 2 Challenges/limitations of microneedles in gene delivery—cont'd

Sl. no.	Challenges	Details	Refs.
4.	Limited scale-up	The scale-up of MN-based gene delivery is another challenge associated with their use. MNs are typically fabricated using microfabrication techniques, which are expensive and time-consuming. The limited scale-up of MNs may limit their commercial viability, particularly in the context of large-scale clinical trials or gene therapy manufacturing	[52]
5.	Stability and degradation	Genetic material can be easily degraded by environmental factors, such as temperature, humidity, and light. Therefore, the stability of the genetic material during the delivery process is crucial	[95]
6.	Immunogenicity and toxicity	The use of MNs for gene delivery may trigger an immune response or cause toxicity. This can be due to the materials used for MNs, the genetic material itself, or the delivery process	[20]
7.	Regulatory approval	The use of MNs for gene delivery may require regulatory approval, which can be time-consuming and costly	[96]

7 Conclusions

MNs have emerged as a promising technology for gene delivery due to their ability to effectively penetrate the skin barrier and deliver genetic material to the underlying cells. This approach can potentially overcome many of the limitations associated with traditional gene delivery methods, such as low transfection efficiency and cytotoxicity. Studies have demonstrated the successful use of MNs for the delivery of various types of genetic material, including plasmid DNA, siRNA, and mRNA. Moreover, MN-mediated gene delivery has been shown to induce robust and sustained gene expression, making it a potential alternative to traditional gene therapy approaches. While there are still challenges to overcome, such as optimization of delivery parameters and minimizing potential side effects, the use of MNs for gene delivery holds great promise for a wide range of applications, including

vaccines, cancer therapy, and regenerative medicine. In conclusion, MNs offer a promising approach to gene delivery that has the potential to revolutionize the field of gene therapy. Ongoing research and development will likely continue to improve the efficiency and safety of this technology, making it an increasingly viable option for clinical use.

8 Future directions

MNs have shown promising results in gene delivery due to their ability to penetrate the skin without causing significant pain or tissue damage. As researchers continue to explore the potential of MNs for gene delivery, here are some potential future directions:

Development of more efficient MNs: Researchers may focus on designing MNs that can deliver genes more efficiently and effectively. This could involve optimizing the size and shape of the needles, as well as the materials used to make them.

Combination with other gene delivery methods: MNs may be combined with other gene delivery methods, such as electroporation or ultrasound, to increase the efficiency of gene delivery. This could lead to more effective and targeted gene therapies.

Clinical applications: As more research is conducted on MNs for gene delivery, there may be an increasing number of clinical applications for this technology. For example, MNs could be used to deliver gene therapies for diseases such as cystic fibrosis or hemophilia.

Personalized medicine: MNs may also play a role in personalized medicine, where gene therapies are tailored to an individual's specific genetic makeup. By delivering genes directly to the skin, MNs could provide a noninvasive and targeted approach to gene therapy.

Moreover, MN-mediated gene delivery has been shown to induce robust and sustained gene expression, making it a potential alternative to traditional gene therapy approaches. Overall, the future of MNs for gene delivery looks promising, and continued research in this area could lead to significant advancements in the field of gene therapy.

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CHAPTER 17

Microneedle-mediated vaccine delivery

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1 Introduction

The SARS-CoV-2 virus is the cause of the COVID-19 pandemic, which has affected both individual and global health as well as the economy, making it the biggest issue of the 21st century [1–3]. Over 190 million infections and over 4 million fatalities have resulted from the virus despite massive efforts to stop its spread [4,5]. It has been shown that vaccination is the most successful strategy for containing the pandemic [5–7]. It is now commonly acknowledged that the world might return to normalcy and get back on track if safe and effective vaccines were made available and a worldwide immunization program was implemented successfully [6,8].

It is commonly acknowledged that vaccination is an easy, efficient, and economical way to stop infectious diseases [1,9]. It is essential to lower the morbidity and death rates connected with these illnesses. Vaccines not only provide people with direct protection, but they also help a particular group develop herd immunity. Thus, the risk of infection among susceptible people is reduced, and the spread of infectious diseases is slowed down. Typically, vaccines contain pathogens that have been inactivated or attenuated, pathogen subunits, or pathogen nucleic acids that encode antigenic proteins [8]. They cause the body to mount an immunological response, which results in the synthesis of particular antibodies and the activation of immune cells. When the same pathogen is encountered again, the immune system remembers it from prior exposure, which strengthens the immune response and increases the pathogen's ability to be eliminated, protecting the person against illness.

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Vaccinations have been shown to be beneficial in avoiding diseases; however, a sizable portion of people either refuse or postpone getting immunized [1,4,6,8,9]. This is partly due to the unpleasant aspects of getting immunized, such as needle anxiety and painful injections [10]. These worries are exacerbated by the fact that, in many situations, syringe injection is still the principal technique for vaccine delivery. Furthermore, syringe-mediated vaccine administration is not without its restrictions. These include the growing danger of accidents from needlesticks, difficulties in preserving vaccine stability, the demand for skilled personnel to administer the shots, and the high expense of storage and shipping since cold chain systems are necessary. These drawbacks highlight the necessity for other vaccination delivery strategies that can allay these worries and increase vaccine accessibility and adoption [8,10]. Researchers have looked into transdermal medication delivery systems as a potential substitute for the shortcomings of conventional vaccination delivery strategies [8]. These systems have advantages over oral or subcutaneous (SC) injections because they transfer medications via the skin in a painless and minimally intrusive way.

In particular, microneedle (MN) technology has drawn a lot of interest from a variety of industries, including sensing, medical diagnosis, and illness treatment [8,10]. Drugs can be delivered locally to target tissues thanks to MNs, which enables long-term therapeutic concentrations and sustained drug release. Through the altering of the microneedle heights, they also provide accurate and efficient distribution to the dermis and epidermis. Furthermore, MNs may be produced and sent for less money, which may eliminate the requirement for cold chain systems during storage and transportation. This makes MNs particularly appealing for vaccination purposes. This chapter provides an overview of the current status of research and clinical applications of microneedle (MN)-based vaccination.

2 Skin-based vaccination

The human skin, being the biggest organ, plays a vital role in providing protection from external harm and microorganisms [11]. It is made up of several layers, such as the SC tissue, dermis, and epidermis, which are each roughly 0.2 mm thick and 2 mm thick, respectively. Different cell types in the skin contribute to its immunoreactivity. In the epidermis and dermis, these include keratinocytes, mast cells, Langerhans cells (LCs), dermal dendritic cells (dDCs), macrophages, and T and B lymphocytes in the lymph nodes connected to the skin [2,7,10] (Fig. 1 left). These elements come together

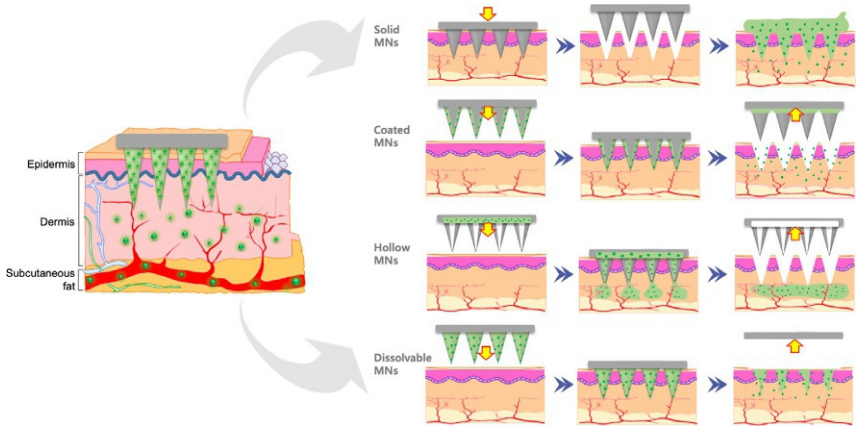

Fig. 1 Schematic illustration of the vaccine delivery mechanism via different types of MNs.

to produce the skin immune system, which is essential for protecting the skin from infection and for producing a particular protective immune response after immunization. By effectively delivering antigens to lymphocytes, antigen-presenting cells (APCs) are essential for the immunological response to vaccinations. dDCs and Langerhans cells (LCs) are the two primary APCs in cutaneous immunity. The presence of Birbeck granules identifies leukemic cells (LCs), which are specialized myeloid cells generated from the bone marrow. LCs travel from the epidermis to the draining lymph nodes in response to antigens, where they stimulate T lymphocytes and start an immunological reaction. Several molecules involved in antigen presentation, including CD1, T cell adhesion molecules, T cell costimulatory molecules, and major histocompatibility complex (MHC) classes I and II, are expressed by LCs, which are very effective at presenting antigens [12].

Apart from LCs and dDCs, the skin contains other immune-competent cells that aid in the beginning and synchronization of immune responses. Mast cells, macrophages, and keratinocytes are some of these cells. The most prevalent cells in the epidermis are called keratinocytes, and they act as a barrier between the underlying tissues and the outside world. In addition to their structural function, keratinocytes are essential for innate immunity. They are able to identify damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs) because they have pattern recognition receptors (PRRs) expressed on their surface [13–16]. Keratinocytes release and express a variety of immunological molecules in

response to identification, including proinflammatory factor receptors, chemokines, cytokines, and antimicrobial peptides [16].

Another significant immune cell group in the skin is the macrophages. These cells are phagocytic, meaning they take up and destroy trash and pathogens [8,17]. Moreover, macrophages release chemokines and cytokines, which are involved in the control of immune responses and tissue regeneration. Macrophages are present in both the dermis and the epidermis of the skin, where they aid in immunological modulation and monitoring. Granulated cells, called mast cells, are widely dispersed throughout the skin. They play a role in the immunological responses against infections and allergy reactions [8,18]. In reaction to particular stimuli, mast cells can release a range of mediators, such as histamine, cytokines, chemokines, and proteases. These mediators can recruit and activate other immune cells, promote inflammation, and contribute to the elimination of pathogens.

These immune-competent cells, which include mast cells, keratinocytes, and macrophages, cooperate to start and manage immunological reactions in the skin. Their interactions and the release of different immune mediators support the skin's overall immunological defense by facilitating the recruitment and activation of other immune cells and regulating the immune microenvironment. In fact, keratinocytes, mast cells, and dermal macrophages—all immunocompetent helper cells found in the skin—have a variety of characteristics that can be used to improve the effectiveness of skin-based vaccinations.

Skin-based vaccination strategies have the potential to improve immune responses by utilizing the characteristics of these immunocompetent helper cells. These cells can draw in and activate other immune cells by secreting immunological mediators, which strengthen the immune response as a whole. Furthermore, cutaneous macrophages have the potential to support efficient antigen absorption and processing as well as immunological control [8,18]. All things considered, maximizing the potential of skin-based vaccination tactics may increase the effectiveness of immunocompetent helper cells in the skin, resulting in improved immune responses and better defense against infectious diseases or other specific disorders.

3 Microneedle-mediated vaccination strategies

Because MN-mediated immunization is a minimally invasive method that lessens pain and increases compliance, it presents a possible substitute for traditional needle injection. MNs' height-adjustable structures allow for

accurate vaccination distribution into the epidermis and dermis layers, boosting skin immune responses. Over the past 10 years, MNs have been the subject of in-depth preclinical studies on vaccine administration [7,8,10].

Based on their structure and capabilities, MNs fall into two primary categories: MN-based drug-device combination systems and MN-based medical devices [12]. MNs that are loaded with vaccines or other pharmaceuticals to enable their delivery are referred to as MN-based drug-device combination systems, whereas MNs employed only for vaccine delivery are known as MN-based medical devices (Fig. 1 right). In this part, we will give an overview of MNs for vaccine delivery, classifying them based on their many categories. A thorough grasp of the many MN technologies used in vaccine delivery is made possible by this classification.

3.1 Solid MNs

Microchannels are created on the skin by applying solid MNs, which can be constructed of silicon [19,20], metal (such as stainless steel) [21,22], or other biocompatible materials. By inserting these microneedles into the skin, transient pores are created. The vaccine can then be delivered topically to the skin through these microchannels, either as vaccine solutions or as hydrogels filled with the vaccine. Solid MNs have been shown in multiple studies to be efficacious in delivering vaccinations, including those for malaria, hepatitis B, and diphtheria toxoid [23–26]. These MN-created microchannels allow the vaccines to permeate the skin and elicit effective immune responses.

In fact, when combined with solid MNs, hydrogel patches have become a viable method for transdermal immunization. Compared to liquid versions, hydrogel patches are easier to use and give the vaccine more stability. An instance of this combination is an MN array by Guo et al. [27] that consisted of 484 monocrystalline silicon solid MNs (150 μm long) on a supporting polymer plate (1 cm^2). It is utilized in conjunction with a hydrogel patch that contains a cholera toxin B (CTB) adjuvant and hepatitis B surface antigen (HBsAg). When compared to the conventional percutaneous immunization, the hydrogel patch vaccination greatly decreased the amount of HBsAg that was needed, which could result in vaccine production cost reductions [27]. Currently, some studies are using external forces like iontophoresis (the use of electric current) and sonophoresis (the use of ultrasound) to enhance the passive diffusion of vaccines through the microchannels created by MNs [28,29]. These techniques can help transport vaccines across the skin barrier, further enhancing the transdermal delivery and efficacy of MN-based vaccination.

3.2 Hollow MNs

As micron-scale syringes, hollow MNs depend on outside pressures to help medicine pass down their channels and get to the right places in the skin. Syringe thrust, pressure differential, and spring elasticity are a few examples of these forces [30–33]. Delivering biological macromolecular medications, such as insulin, nucleic acids, and vaccinations, has demonstrated significant promise for hollow MNs [8,34,35]. For instance, van der Maaden et al. created hollow MNs for the delivery of inactivated polio vaccine (IPV) by etching fused quartz capillaries using the fused fluorosilicic acid process [36]. The findings showed that, as compared to a single immunization with identical dosages of the vaccine, the immunological response caused by hollow MN-mediated intradermal (ID) injection of IPV was comparable to that induced by conventional SC injection, which boosted the immune response. Compared to the use of adjuvants, these results revealed that receiving numerous immunizations could boost immune responses and lower vaccine costs. However, as a means of enhancing adjuvant qualities, vaccination durability, and antigen release, nanoparticle (NP) vaccines are drawing more and more interest [37,38].

In addition, De Groot and Du et al. developed a poly (lactic-*co*-glycolic acid) (PLGA) NP vaccine encapsulating ovalbumin (OVA) and a TLR3 agonist poly(I:C) adjuvant for ID administration using hollow MNs [30]. The NP vaccination produced strong CD8+ T cell responses and moderate CD4+ T cell responses when administered intraperitoneally to mice. It is interesting to note that using cationic PLGA NPs increased the immune response more than anionic PLGA NPs, maybe because of the interaction between the NPs' positive charges and the negatively charged surface of the dendritic cell (DC). The positive charges increased the contact between the particle and the cell membrane, which improved cell phagocytosis. Similar to this, Niu et al. created PLGA NP vaccines and used hollow MNs and a syringe pump to administer them. The vaccinations contained OVA antigen and TLR4/TLR7 agonists as adjuvants [32]. Delivery of OVA NPs through hollow MNs produced equivalent amounts of IgG1 antibodies and higher levels of IgG2a antibodies compared to intramuscular (IM) injection, indicating a balanced Th1/Th2 immune response. Moreover, a more robust humoral and cellular immune response was generated by the NP vaccination administered via hollow MNs.

All things considered, NP vaccines administered via hollow MNs have benefits in terms of maintaining antigen release, making use of the lymphatic

system, and encouraging a potent and well-balanced immune response. These results demonstrate the possibility of effective transdermal immunization with NP-based tactics combined with MNs.

3.3 Coated MNs

The process of creating coated microneedles (MNs) involves coating the surface of solid MNs with a vaccination solution or dispersion. The vaccine coating progressively separates from the surface of the MNs after skin implantation and moves via the interstitial fluid into the dermis. After that, the vaccine is absorbed by dDCs or Langerhans cells (LCs), which triggers immunological reactions and results in vaccination.

A five-tip MN array was created by Kines et al. using stainless steel sheets, and it was repeatedly coated with a vaccination solution that contained plasmids encoding respiratory syncytial virus antigens and HPV pseudovirus [39]. Studies conducted in vivo revealed that IM injections or vaccination with coated MNs produced comparable levels of neutralizing antibodies, proving the viability and efficacy of coated MNs for immunization. Furthermore, the discomfort of needle injections was lessened when MNs were used. The vaccine coating on the MNs prevented the necessity for cold chain storage and transportation by maintaining antigen stability at room temperature. For the purpose of creating coated MNs, polymeric materials like poly (lactic acid) (PLA) have also been investigated. To stimulate mice's immune systems, Choi and Cha et al. created PLA MNs coated with live vaccinia virus [40]. Following immunization, the MNs produced neutralizing antibodies and secreted more IFN-γ, showing the activation of both humoral and cellular immunity. This was achieved by soaking them in a coating solution containing the vaccine. By adding polymers like trehalose and polyvinyl alcohol (PVA) to the coating solution, the durability of the vaccine coated on MNs can be improved, enabling better preservation during preparation and storage.

Silk fibroin is frequently used as a coating material to shield vaccinations from high temperatures [41,42]. A silk fibroin MN patch was created by Kaplan and associates to administer vaccinations against *Shigella*, *Clostridium difficile*, and influenza [43]. The MNs with the antigen coating showed improved resistance to temperature changes, making cold chain storage unnecessary. Additionally, it has been demonstrated that coated MNs improve immunological responses to antigens [44,45]. When DNA vaccines were applied topically using coated MNs instead of traditional syringe

injection, Zhang et al. showed enhanced immunogenicity and antigen expression [44,46]. A longer length of protein expression and a markedly higher production of the encoded antigen were the outcomes of uniform plasmid DNA dispersion on the MN surface.

Lastly, coated MNs provide benefits such as increased immune response activation, stability augmentation, and sustained vaccination release. MNs have been coated with a variety of materials, including polymeric and inorganic compounds, indicating their potential for effective transdermal immunization.

3.4 Dissolvable MNs

Dissolvable microneedles (MNs) have drawn interest in the delivery of vaccines because they may allay the safety concerns related to insoluble MNs composed of metals or silicon. Water-soluble polymers like sucrose, maltose, hyaluronic acid, CMC, PVP, and PVA can be used to create these dissolvable MNs, which have remarkable mechanical strength, water solubility, and biocompatibility [8,10,12]. These MNs release encapsulated vaccines by skin dissolution and degradation, which triggers an immunological response.

Several strategies have been investigated by researchers to improve the immunogenicity and stability of vaccines based on dissolvable MN. Various ways have been explored to enhance vaccine stability, including the use of excipients, process optimization, lyophilization, and nanotechnology. It has been discovered that excipients, including arginine/heptagluconate, help in protein folding and prevent protein aggregation, preserving vaccine efficacy over time [47]. MNs that can be stored at high temperatures without noticeably losing their vaccination efficacy have been created using the process of lyophilization, which involves removing water molecules from the proteins to increase their stability.

Adjuvants are frequently used to boost the immune response to vaccines based on their immunogenicity [48]. Adjuvant effects are possible for certain MN materials, such as chitosan and other biodegradable biomaterials. It has been demonstrated that chitosan-based MNs improve chitosan's adjuvant qualities and lower the quantity of antigen needed for immunization [49]. Additionally, vaccination immunogenicity may be impacted by the molecular weight of the MN components. High molecular weight hyaluronic acid (HMW-HA) may have immunosuppressive and antiinflammatory effects, while low molecular weight hyaluronic acid (LMW-HA) has been shown to stimulate immune cells and function as an adjuvant [50].

Novel approaches to improve vaccination immunogenicity have also been studied [1,51]. For instance, a melanin-mediated cancer immunotherapy patch raises the local temperature by inducing the release of inflammatory chemicals, synthesis of immunogenic substrates, and absorption of APCs [52]. This strategy slowed the growth of tumors and improved anticancer vaccination. Additional research has looked into the use of combination adjuvants, complex MNs, dendritic cell-targeting ligands, and nanovaccines to enhance vaccination-induced immune responses [8]. Programmable burst release methods have been devised so that a single MN patch can be used for many vaccinations. Using soluble MNs with varying rates of degradation or molecular weights, these systems enable fine control over the release period. Clinical vaccinations have been used in animal research, and the results have been encouraging.

Researchers have been investigating the use of dissolvable MNs for self-vaccination and to increase immunization rates worldwide in light of the COVID-19 pandemic [8,10]. Dissolvable MNs have been shown in studies to be an effective means of delivering SARS-CoV-2 subunit vaccines, eliciting strong antibody responses, and inducing T-cell responses that are on par with injectable techniques. Dissolvable MNs present a promising approach to vaccine distribution overall, with possible advantages like greater immunogenicity, improved stability, safety, and the capacity to facilitate self-vaccination and minimize interpersonal interaction during pandemics. To guarantee the broad implementation of these systems and optimize them for a variety of vaccines, more studies and developments are required. Clinical trials have already demonstrated the efficacy of a number of coated and dissolvable MNs [53–55].

4 Clinical trials of MN-mediated vaccination

A 2009 study by Van Damme et al. investigated the use of MNs in adult Europeans to administer a low-dose influenza vaccination [56]. The outcomes demonstrated that, when compared to full-dose IM vaccination, the immune responses elicited by the MN-delivered vaccine were comparable. Hollow MNs were thoroughly investigated for vaccine delivery after this significant event. Hollow MNs have been used in clinical trials to administer vaccinations, including rabies, varicella-zoster, hepatitis B virus (HBV), and inactivated poliovirus (IPV) vaccines [8].

In order to administer influenza vaccinations, coated and dissolvable MNs were also created as drug-device combination products. Searching

clinicaltrials.gov with the terms "microneedle" and "vaccine" turned up a total of 18 clinical trials pertaining to MN-mediated immunization. Sheng et al. [12] reviewed 16 of these trials after they were finished (Table 1). While therapeutic and preventative tumor vaccines are still in the preclinical stages of research, all of the clinical trials were solely focused on vaccination against viruses. The clinical data that are now available shed light on the acceptability, stability, safety, and effectiveness of MN-mediated immunization. The chapter does not, however, elaborate on the particular discoveries made in these fields.

5 Conclusion and perspective

Significant efforts have been undertaken to enhance MN-based vaccine administration since the introduction of MNs in 1998. Reducing vaccination doses, raising immunogenicity—the capacity to elicit an immune response—improving vaccine stability, and boosting patient compliance are some of these initiatives. Three important characteristics of the MN-mediated vaccines were examined in clinical trials: stability, effectiveness, and safety. Out of the three aforementioned factors, the first two are essential requirements for the continued application of MN-mediated vaccination in clinical settings. Although the safety and efficacy of commercially available hollow microneedles (MNs) for vaccine delivery in human subjects have already been assessed, MNs and vaccines are not integrated into products; instead, MNs and vaccines are utilized as injectable devices Sheng et al. [12].

MN-based tactics provide for the regulated release of vaccines, making immunization schedules adaptable. Promising results have been observed in clinical trials involving some MNs for vaccine delivery. Research is required to investigate materials that guarantee biosafety and increase performance in order to further improve MN-based vaccine delivery. It is important to enhance the mechanical qualities of MN materials to enable successful transdermal distribution without breaking them. Furthermore, a complete assessment of biocompatibility needs to be done in both in vivo and in vitro environments. To successfully commercialize MN-based vaccine delivery, the hurdle of successful scale-up manufacturing must be defeated. Ensuring homogeneity of MN parameters and vaccine loading levels during large-scale production should be the main focus of investigations.

Improving immunization coverage requires accurate vaccination records. For instance, when mixed with a vaccine and subjected to near-infrared light, McHugh et al.'s produced MNs loaded with near-infrared

Table 1 An overview of the representative clinical trials from clinicaltrial.gov that use microneedles to administer vaccines.

Microneedle type	Phase	Study target	Vaccine strategy	Subjects	Clinicaltrials.gov identifier
Hollow MNs	Not applicable	Study safety and immunogenicity of Bacillus Calmette-Guerin (BCG) delivery via novel MicronJet 600 device	BCG vaccination	Age: 19 years to 35 years healthy adults Sex: all sexes $N=15$	NCT04064554
Dissolvable MNs	1	Evaluating the safety, reactogenicity, and acceptability of placement of a placebo microneedle patch	Vaccination	Age: 6 weeks to 24 months healthy child Sex: all sexes $N=33$	NCT03207763
Hollow MNs	3	To compare the safety and immunogenicity of ID HBV by this novel device with IM injection	HBV with imiquimod	Age: $\geq$ 21 years patients on renal replacement Sex: all sexes $N=94$	NCT02621112
Coated MNs	Not applicable	To investigate the inertness and biocompatibility of uncoated nickel and gold- or silver-coated microneedles	Vaccination	Age: 18 years to 60 years adults with proven nickel allergy Sex: all sexes $N=15$	NCT02995057
Hollow MN	2,3	To assess in recipients of hematopoietic stem cell transplants, the safety and effectiveness of the new ID live-attenuated varicella-zoster vaccine	Varicella-zoster vaccine	Age: 18 years to 60 years adults undergoing allogeneic hemopoietic stem cell transplant or HLA identical sibling donors Sex: all sexes $N=100$	NCT02329457

Continued

Table 1 An overview of the representative clinical trials from clinicaltrial.gov that use microneedles to administer vaccines—cont'd

Microneedle type	Phase	Study target	Vaccine strategy	Subjects	Clinicaltrials.gov identifier
Hollow MNs	3	To evaluate the immunogenicity of oral poliovirus vaccine (OPV) and a sequential dose of fractionally inactivated poliovirus vaccine (f-IPV) administered by MNs	IPV	Age: 6 weeks to 7 weeks healthy child Sex: all sexes $N=1206$	NCT01813604
Hollow MNs	2,3	To evaluate the impact of skin routes of immunization	Influenza vaccine	Age: 18 years to 45 years healthy adults Sex: all sexes $N=60$	NCT01707602
Dissolvable MNs	1,2	To evaluate the immunogenicity and safety of a measles rubella vaccination MN (MRV-MN) patch in adults, toddlers primed with MRV, and infants naïve to MRV	MRV	Age: 9 months to 40 years healthy child or adults Sex: all sexes $N=225$	NCT04394689

light can be used as long-term on-person immunization records [57]. A number of conditions must be satisfied for MN-mediated vaccine administration to be commercialized successfully, including increased patient compliance, cost savings, and worldwide accessibility [7,8,10]. In the end, a few fundamental conditions must be realized for MN-mediated vaccine administration to be successfully commercialized, which would enable it to serve as a more widely available vaccination technique while greatly enhancing patient compliance and reducing expenses.

Author contributions

T.T. N and G.V.·V conceived the work; G.V·V wrote the first draft of the chapter. All authors revised the manuscript.

Competing interests

The authors declare no competing interests.

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CHAPTER 18

Advancements and applications of 3D-printed microneedles for drug delivery

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1 Introduction

3D printing, an additive manufacturing technique, converts computer-aided 3D design model input to tangible objects like prototypes and layer-by-layer architectural design [1]. In 3D printing, the adaptive manufacture of organ and tissue regeneration, medical devices like prostheses and implants, and personalized medications like 3D-printed tablets. For the treatment of epilepsy, the orally administered drug Spritam (Levetiracetam), which is called the first 3D-printed pill, was approved by USFDA in 2015 [2].

There are several uses for 3D printing since each process has different biocompatibilities, resolutions, output volumes, and cost-effectiveness trade-offs. The conception of a variety of laboratory instruments for point-of-care and clinical applications, such as wound healing, organs-on-a-chip devices, embryology and fertility research, tissue engineering, isolation of tumor cells, cancer research, and stem cell research, can be made possible by using biomaterials in 3D-printing processes, as well as nanoscale and microscale 3D printing [3].

Microneedle arrays are small needles that carry drug molecules through the skin and bypass the stratum corneum by forming small pores and reaching the systemic circulation. The materials used for microneedle fabrications are ceramics, polymers, silicones, and metals [4]. The production of custom-shaped microneedles using 3D-printing technology has attracted much attention recently. The height of the microneedles ranges from 25 to 2000 μm. Microneedles help monitor and diagnose the disease by inserting microneedles into the skin, which creates pores of micron size in the

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epidermis to collect interstitial fluid and deliver drug molecules into microcirculation through diffusion [5].

The advantages of microneedles are that they can penetrate the skin and cross the stratum corneum, avoid puncture of nerve fiber and blood vessels because of the size of the needles, are patient-friendly, and enable access to reach microcirculation in the skin, and are painless on insertion [6]. Recently, the fabrication of microneedles using 3D print has played a vital role in manufacturing specific properties of needles like tip-radius, needle thickness, needle height, and density by high resolution and reproducibility. The fabrication of 3D-printed microneedles using dissolvable polymers has biodegradable and biocompatible properties; hence, it is a promising tool for increasing the functionality and efficacy of microneedles [7].

2 Types of fabrication techniques in 3D-printed microneedles

The fabrication techniques of microneedles are divided into three types: Vat Polymerization, Material Extrusion, and Powder bed Fusion according to ISO/ASTM 52900 for the addictive manufacturing process. The different types and their application are described in Table 1.

2.1 Material extrusion

A 3D object is created using material extrusion systems, which selectively dispense the printing ink containing a mixture of excipients, drug molecules, and binder via an orifice or nozzle. To create a 3D structure, in this type of 3D printing, the ink disperses via a nozzle. It is deposited layer by layer using computer-spatially controlled processes based on the kind of method involved and printing material utilized; the material extrusion is classified into two types, such as fused deposition modeling (FDM) and pressure-assisted microsyringes (PAMS).

2.1.1 Pressure-assisted microsyringes (PAMS)

The 3D object is fabricated in PAMS using semi-liquid ink, which extrudes via a microsyringe. To eject the printing material from the microsyringe, the compressed air force to obtain reproducibility of the object, the solid loading, and additives affect the characteristics of the printing material. Using solvents, such as those found in INKJET systems, is the main drawback of PAMS [26].

Table 1 3D-printing technologies used for fabricating microneedles.

Method of 3D printing	Combined technology	Type of microneedles	Materials used for fabrication	Application of microneedles	References
FDM	Chemical etching	PLA microneedles	PLA and Sodium hydroxide	Drug delivery	[8]
FDM	Chemical etching	PLA microneedles	PLA and Potassium hydroxide	Drug delivery	[9]
FDM	–	Polymeric microneedles	PVA/PVP	Drug delivery	[10]
LCD	–	Hollow microneedles	Biocompatible resin	Insulin delivery	[11]
SLA	Inkjet printing	Polymeric Microneedle	Biocompatible resin	Anticancer delivery	[12]
SLA	–	Hollow microneedles	Biocompatible class I resin	Drug delivery	[13]
SLA	Inkjet printing	Polymeric microneedles	Biocompatible class I resin	Insulin delivery	[14]
SLA	Isotropic shrinkage	Polymeric microneedles	RGD 720 resin and PVP	Drug delivery	[15]
TPP	–	Hollow microneedles	Ormocer	ISF extraction and drug delivery	[16]
TPP	–	Solid microneedles	Femtosecond laser pulses	Drug delivery	[17]
TPP	Micromolding	Polymeric microneedles	Polyethylene glycol 600 diacrylate	Drug delivery	[18]

Continued

Table 1 3D-printing technologies used for fabricating microneedles—cont'd

Method of 3D printing	Combined technology	Type of microneedles	Materials used for fabrication	Application of microneedles	References
TPP	Iron sputtering deposition	Magnetic microneedles	Photoresist	Tissue engineering, drug delivery, and single-cell analysis	[19]
CLIP	Dynamic printing	Polymeric microneedles	Biocompatible polymers	Drug delivery	[20]
Microstereolithography	–	Microneedle splints	3DM cast resin	Drug delivery	[21]
Microstereolithography	Inkjet printing and micromolding	Polymeric microneedles	eShell-200 and Gantrez AN-169	Quantum dots delivery	[22]
Microstereolithography	Pulsed laser deposition	Solid microneedles	eShell-200	Drug delivery	[23]
Microstereolithography	–	Polymeric microneedles	Polyethylene glycol 600 diacrylate	Drug delivery	[24]
SLM	–	Hollow microneedles	Stainless steel	Drug delivery	[25]

2.1.2 Fused deposition modeling (FDM)

In fused deposition modeling (FDM), the hot-melt extrusion of the thermoplastic polymer at the print head method is used for 3D printing. The polymer filament is concurrently extruded and deposited on the print station as the print head moves following the pattern in the 2D slice. In FDM, different polymer filaments can be used a single process. For example, the filament used for the main object and the filament used for fabricating the supporting object differs, facilitating the prototype's postprocessing. Once the first layer solidifies and cools down, the same process is used to create the second layer, making the final 3D-printed prototype, as shown in Fig. 1 [1].

In a study, the PLA microneedles were developed using fused deposition modeling, but the needles formed with FDM have poor adhesion within PLA layers. Hence, the cylindrical shape of PLA is fabricated using FDM 3D printing. With chemical etching, microneedles from cylindrical shapes were fabricated by allowing the cylindrical shape of PLA polymer to be immersed in potassium hydroxide at different time points. The tip diameter of the microneedles ranges from 1 to 55 μm, and the width of the microneedles was reduced by half of the original size. The fabrication of microneedles improved the accuracy of drug loading [27].

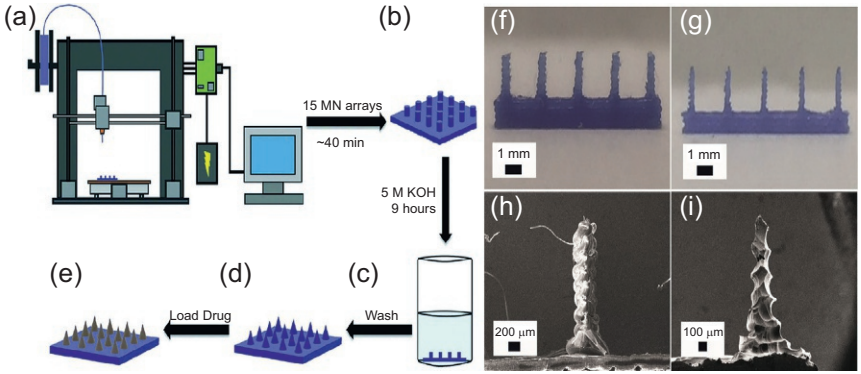

Fig. 1 (A)–(E) Schematic representation of steps involved in the fabrication of microneedles using chemical etching and FDM 3D printing; (F) optical images of FDM-printed microneedles; (G) optical images of chemical etching microneedles in potassium hydroxide solution; (H) SEM images of FDM-fabricated microneedles; and (I) SEM images of chemical etching microneedle in KOH solution. *(Reprinted with permission from M.A. Luzuriaga, D.R. Berry, J.C. Reagan, R.A. Smaldone, J.J. Gassensmith, Biodegradable 3D printed polymer microneedles for transdermal drug delivery, Lab Chip 18 (8) (2018) 1223–30. Available from: https://pubmed.ncbi.nlm.nih.gov/29536070/.)*

In this chapter, the production of microneedles was simplified by the preparation of negative molds in FDM 3D print, and it was developed by controlling the process parameters through experimental design. In 3D printing, the factors affecting reproducibility are printing material interaction, layer height, and path width. Once the optimization and process parameters were fixed, the microneedles were fabricated using PVA/PVP polymer with the API galantamine hydrobromide. The prepared microneedles were characterized by analyzing the % drug release, drug loading, and the interactions between polymer, drug, and printing material using an artificial membrane called Start-M. As a result, the prepared microneedles release the maximum amount of drug molecule; furthermore, the prepared formulation examined permeation efficiency through the skin by ex vivo studies. This technology is a novel drug delivery system for enhancing the transdermal delivery route [28].

2.2 Vat photopolymerization

Vat photopolymerization is a common additive manufacturing (AM) method that uses a digital light processing (DLP) light engine to produce a patterned light source. It should be noted that printing extreme-size objects is complex, and choosing the printing conditions has traditionally relied heavily on repeatable trial-and-error experimentation. It is a widely used method for high resolution and speed of the printing process. There are three types of printing techniques in vat polymerization: digital light processing (DLP), stereolithography (SLA), and two-photon polymerization (TPP). DLP printing can cure the projection simultaneously, whereas SLA and TPP cure only one point simultaneously, which helps in rapid printing speed and high printing resolution. The DLP printing system's fundamental component is a digital micromirror device (DMD). A lattice of micromirrors used in the construction of DMD chips can either help or hinder light reflection, depending on the angle at which they reflect. Then, the micromirror array is loaded in the patterned projection, and the light pattern to the bottom of the liquid tanks is projected [29].

2.2.1 Stereolithography (SLA)

In stereolithography, the photopolymerization method is used to develop a 3D object, in which liquid resin is used for fabrication by passing a UV laser beam in the vat. UV laser helps trace the object's pattern on the liquid resin surface, and later, the liquid resin is dried and solidified upon UV exposure. After completion of the first layer, the fresh resin is allowed to cover the first

layer by bringing down the build platform. The next layer of the object begins by inducing UV-light photopolymerization. Once the 3D object is fabricated on the build platform, the uncured resin is washed using a solvent, and to solidify, the prototype is exposed to UV light.

The SLA 3D print technique has recently been the most useful method for fabricating different microneedle types. However, the main drawbacks are more fabrication time and high production cost. Hence, it is used to manufacture master molds, which can be used several times to create female molds, as shown in Fig. 2. As a result, it achieves a production rate of

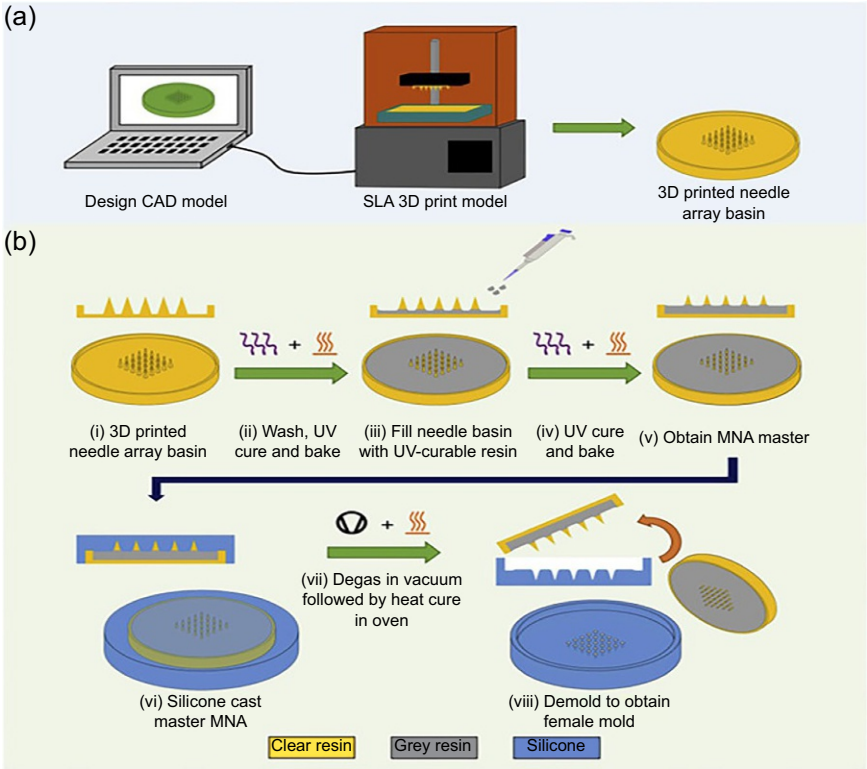

Fig. 2 Schematic representation of print and fill method of microneedle fabrication. (A) CAD design of microneedle array and fabrication using SLA 3D printer and (B) Steps involved in the fabrication of microneedles in a two-step fabrication process. *(Reprinted with permission from K.J. Krieger, N. Bertollo, M. Dangol, J.T. Sheridan, M.M. Lowery, E.D. O'Cearbhaill, Simple and customizable method for fabrication of high-aspect ratio microneedle molds using low-cost 3D printing. Microsyst. Nanoeng. 5 (1), (2019) Available from: https://pubmed.ncbi.nlm.nih.gov/31645996/. Copyright 2019, Springer Nature.)*

microneedles, is less expensive, and increases the production of microneedles in the future. Several types of microneedles are fabricated using SLA 3D printing as follows.

Solid microneedles

SLA 3D-printing technology is the most extensively used method in solid microneedles. Ochoa et al. reported the first solid microneedles fabricated using SLA 3D print. They have developed a master mold fabricated with SLA 3D printing, and a female mold of agarose gel was fabricated using a master mold. The structure of microneedles fabricated with SLA remains unchanged when female mold of agarose gel was fabricated using isotropic shrinkage. The dried agarose gel was then utilized as a mold to create microneedles from polydimethylsiloxane (PDMS) polymer. They fabricated PVP-based microneedles using PDMS with a tip of 9.6 μm helps to penetrate porcine skin [30].

For the first time, the print and fill method was introduced by Krieger et al. to create a microneedle master mold. The photocurable resin was poured into the microneedle base to develop a different height of microneedles. The microneedles get shorter when the more resin is poured into the basin, and vice versa. The master microneedle was used to create silicone molds after UV light curing using the micromolding technique. This study used a silicone mold to fabricate MNs utilizing various materials and methods, such as PLA-thermal molding and carboxymethyl cellulose (CMC)-solvent casting [31].

Coated microneedle

For insulin-coated microneedles, the combination of SLA and inkjet 3D printing is developed by Pere et al. The microneedles were fabricated by SLA and inkjet printing and by coating insulin with stabilizers like mannitol, trehalose, and xylitol in 1:5 ratios. The picolitre pipetting method achieved the accurate amount of insulin (350 μg) coated on the microneedles' surface. The release kinetics of insulin shows that around 57%–69% in 5 min, and about 90%–95% of insulin is delivered in an hour through the skin [32].

Hollow microneedles

In SLA 3D printing, the photopolymerization method was used to fabricate hollow microneedles with high-resolution printing. Based on the CAD model, the laser beam polymerizes materials in a layer-by-layer prototype. For activating the polymerization process, a Galvano mirror is used for

navigating the laser beam to the resin vat based on the g-code. After completion, the printed object is washed with isopropyl alcohol to remove excess resin and exposed to UV light to solidify and cure the printed prototype [33].

2.2.2 Two-photon polymerization (TPP)

The methods of fabrication followed in TPP are temporal and spatial photon overlap-inducing photochemical reactions. In TPP, the fabrication process involves three main steps: initiation, propagation, and termination. A femtosecond laser beam focused on the tiny volume of the photosensitive resins by a high numerical aperture microscope objective initiates the process by cleaving chemical bonds with photoinitiator molecules. The photoinitiators are stimulated in this stage, followed by their breakdown into radical species. The propagation process involves the interaction of monomers and radical species to get monomer radicals. In the termination process, the polymerization ends by combining two monomer radicals, as shown in Fig. 3.

Doraiswamy et al. fabricated hollow microneedles using the two-photon polymerization (TPP) method. These hollow microneedles were made from organic-inorganic hybrid materials. The organic portion comprises ceramics or glasses, and the inorganic portion is a mixture of biocompatible organic monomers and photosensitive siloxanes with additives, such as a photoinitiator. These substances are called organic-modified ceramics or Ormocer [34].

2.2.3 Continuous liquid Interface production (CLIP)

A UV-transparent window is swapped out for an oxygen-permeable window in the modified SLA design of CLIP. A continuous series of UV pictures are projected via an oxygen-permeable, UV-transparent window placed below a liquid resin bath as part of the CLIP process, as shown in Fig. 4. The uncured liquid layer between the surface of the cured section and the oxygen-permeable window generates a dead zone that prevents the resin from curing and adhering to the window. Contrary to SLA, CLIP no longer produces objects layer by layer. However, it constantly and quickly produces object layer since the separation and realignment processes are eliminated. As a result, no stacking artifacts are visible on the smooth surfaces of the objects created by CLIP [35].

According to Johnson et al., CLIP can fabricate microneedles from various polymers in various geometries and morphologies, such as arrowhead, turret, pyramidal, and tiered microneedles. The array of microneedles was

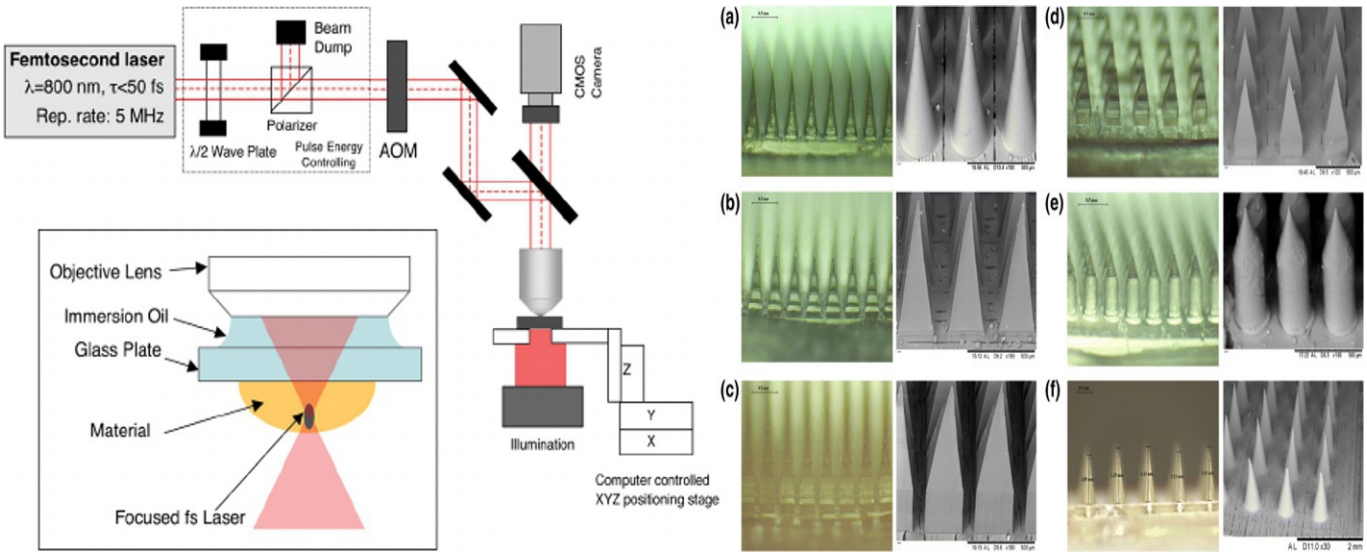

Fig. 3 Schematic representation of the two-photon polymerization process. Light microscopic image *(left)* and scanning electron microscopic image of microneedle *(right)* designs prepared with dissolving formulation (A, D1 to F, D6). *(Reprinted with permission from V.F. Paz, M. Emons, K. Obata, A. Ovsianikov, S. Peterhänsel, K. Frenner, et al. Development of functional sub-100 nm structures with 3D two-photon polymerization technique and optical methods for characterization, J. Laser Appl. 24 (4) (2012) 042004. Available from: https://lia.scitation.org/doi/abs/10.2351/1.4712151. Copyright 2012, Laser Institute of America. Reprinted with permission from A.S. Cordeiro, I.A. Tekko, M.H. Jomaa, L. Vora, E. McAlister, F. Volpe-Zanutto, et al. Two-photon polymerisation 3D printing of microneedle Array templates with versatile designs: application in the development of polymeric drug delivery systems, Pharm. Res. 37 (9) (2020). Available from: https://pubmed.ncbi.nlm.nih.gov/32856172/. Copyright 2020, Springer Nature.)*

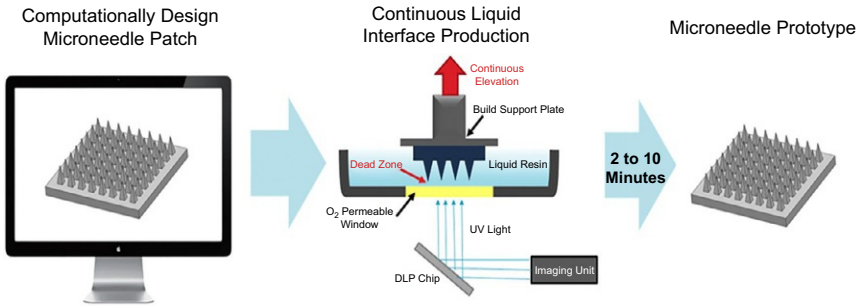

Fig. 4 A schematic illustration of the fabrication process of microneedles using the continuous liquid interface production (CLIP) method. *(Reprinted with permission from A.R. Johnson, C.L. Caudill, J.R. Tumbleston, C.J. Bloomquist, K.A. Moga, A. Ermoshkin, et al. Single-step fabrication of computationally designed microneedles by continuous liquid Interface production, PLoS One 11 (9) (2016) e0162518. Available from: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0162518. Copyright 2012, Laser Institute of America.)*

accurately fabricated in less than 90 s. The microneedles fabricated using CLIP helps in the rapid delivery of rhodamine loaded in microneedles and better skin permeation examined using ex vivo murine skin [36].

2.2.4 Microstereolithography

In the microstereolithography method, the 3D microstructures are fabricated with rapid prototyping and high resolution of the object than the SLA fabrication method, also known as the direct light projection technique. The fabrication process is categorized into three stages: scanning, projection, and submicrometer resolution. For the fabrication of microneedles, projection-based microstereolithography is used.

Similar to stereolithography, for the fabrication of prototypes, projection-based microstereolithography is used for the polymerization of resin instead of UV light for scanning layer-by-layer vat, which illuminates 2D slice images to cure the resin. A digital micromirror device (DMD) manages a sequence of 2D slices to create consecutive dynamic mask patterns for projecting a micro-sized laser beam on the resin surface. Then, the prototype solidified into their respective shape on exposure to light. Later, the fabrication of the first layer completes the next layer formed on the built platform by immersing the prototype into a resin vat [37].

2.3 Powder bed fusion (PBF)

In powder bed fusion (PBF), thermal energy fabricates a 3D prototype from powder materials. Two techniques are used for fabricating PBF-based microneedles: selective laser sintering (SLS) and selective laser melting (SLM). The PBF printing process, SLS, is widely employed in the pharmaceutical industry. SLS creates three-dimensional structures by sintering selective powders using high-energy lasers. This system includes a spreading roller (re-coater), a build platform, a powder build chamber, a powder feed chamber, and a high-power laser source. SLS creates three-dimensional structures by selectively exposing powdered, highly divided particles to high-energy lasers. This system includes a spreading roller (re-coater), a build platform, a powder build chamber, a powder feed chamber, and a high-power laser source. The SLS technique comprises a powder feed chamber, build platform, spreading roller, powder build chamber, and laser source with a scanning mechanism and high powder.

To create a 3D prototype, CAD design is imported to an SLS printer. The printing process starts when the information is sent to the printer. The powder is first fed into the powder feed chamber and spread onto the build platform by the spreading roller. Then, by the CAD design, a high-power laser beam is focused on the uniformly distributed layer, with particles sintering and solidifying the powder to produce a two-dimensional layer. A second layer is then distributed on top of the first layer and sintered to it using a laser once the construction platform has descended to a height of layer thickness. This process will repeat until the 3D prototype is formed. Once the 3D prototype is completed, the prototype is taken out and removes the excess powder retention [38].

The powder bed fusion (PBF) process parameters include temperature, bed thickness, particle density, size distribution, and shape. The more significant variance in particle size distribution may significantly segregate the sintering process. To accomplish compelling sintering of particles and uniform spreading, the powder must have the ability to flow and a particle size range of 58–180 μm. This process has various disadvantages, such as being challenging to process for heat-sensitive drugs and the inability to reuse powder, significantly affecting the prototype's mechanical properties [39].

2.3.1 Selective laser melting (SLM)

Gieseke et al. first fabricated a hollow microneedle using the microselective laser melting method from 316 L powder stainless steel alloy. In this method,

the hollow microneedles are fabricated using a 19.4 μm diameter of the laser spot and particle size ranging from 5 to 25 μm. Because of powder distribution, the surface of microneedles is granular. Moreover, high-energy input-induced powder adhesion along the wall occluded some areas of hollow MNs. In this study, the smooth-surfaced hollow microneedles are fabricated using SLM 3D printer [40].

The advantage of this method is that different types of materials are used while manufacturing the properties of the materials can be tuned by the manufacturer and be cost-effective. On the other hand, metallic alloys are among nickel, titanium, stainless steel, and aluminum utilized in powder-based printing techniques. Therefore, it is crucial to consider biocompatibility of these metals. Moreover, the SLM process uses laser-guided powder melting to fabricate the final product. This may result in a microscopic anisotropy of the material along the direction of construction, leading to the production of breakable items. MNs made using SLM have undesirable rough surfaces because, as previously mentioned, powders are used as the raw material. This necessitates an additional polishing procedure. Various postprocessing techniques can be used to reduce the surface roughness of SLM-produced objects. This comprises vibratory finishing, drag finishing, chemical polishing, machining, shot peening, grinding, and electropolishing [41].

3 Factors affecting 3D-printed microneedles

There are several criteria to follow for an effective transdermal drug delivery system, such as accurate dimensions and design, insertion depth of the needles without breaking, and using biocompatible materials.

3.1 Selection of materials

The materials fabricating microneedles should have high penetration capacity through biological barriers, be compatible with drug molecules, and be easily manufactured. The parameters affecting the materials' properties include tensile strength, biocompatibility, drug loading, and stability of microneedles [42]. For fabricating microneedles, the biocompatibility of the materials and safety are essential factors. To develop a transdermal drug delivery system using microneedles, AM technologies used various fabrication materials with toxic-free biodegradability, scalability, biocompatibility, and mechanical strength [43]. The commonly used resins for SLA 3D printing are methacrylate-based resins [44], and for DLP microneedles,

acrylate-based resins, polyethylene glycol diacrylate, and poly-propylene fumarate were used [45,46]. For CLIP 3D-printed microneedles, FDA-approved materials are used, such as polycaprolactone trimethacrylate and polyethylene glycol dimethacrylate with diphenyl (2,4,6-trimethyl-benzoyl-)phosphine oxide [47]. In TPP 3D-printed microneedles, the Ormocer or acrylic is used [48,49].

3.2 Precision of 3D printing

Despite of 3D-printing technique, a thorough investigation of the impact of significant process parameters is necessary to obtain the desired output quality. To improve geometrical and dimensional accuracy and surface quality, one of the crucial criteria for obtaining the desired product quality is the optimization of process parameters [50]. Printer resolution is one of the essential factors in microneedle production for getting the required microneedle design and producing sharp microneedle tips. Both the device's software and hardware have an impact on print resolution. The mesh density influences the printing accuracy, in which fine mesh density will print more accurately than uneven mesh density [51].

Layer thickness is one of the process variables that significantly impacts the printing resolution in the z-axis direction. Due to the prominent "staircase" phenomena, a curved surface can be created, as shown in Fig. 5. The accuracy of the finished sample decreases in geometry as layer thickness increases, and the "staircase" phenomenon becomes more apparent [53].

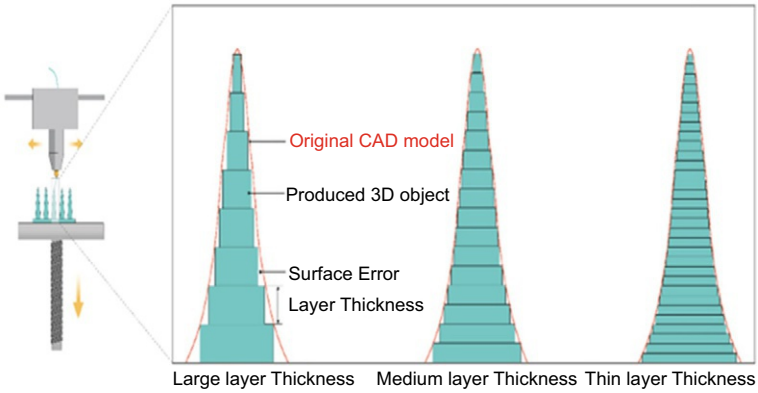

Fig. 5 Schematic representation of different layer thicknesses of staircase phenomenon [52].

3.3 Microneedle design

The geometry of microneedles, including microneedle base, height, shape, tip diameter, and interspace, substantially influences some of the essential microneedle features, such as insertion of microneedles and penetration behavior, capacity to pierce the skin, or amount of drug administration, regardless of the production method [54]. In dermal tissues, the penetration of microneedles through the epidermal layer of skin depends on the length of the microneedles ranging from 150 to 2000 μm [55]. The size of microneedles is a crucial feature in fabrication. If the length of microneedles increase too much the tensile strength get compromised and microneedles can easily break on application of little shear. The noninvasive devices are developed for patient monitoring and diagnosis through microneedles, in which the height of the microneedles must be 900 μm when fabricated for extracting interstitial fluid and monitoring exogenous materials or biological markers [56].

The tip or shape of the microneedles fabricated through 3D printing may also differ. Not all 3D-printing technology can fabricate all these microneedle shapes, as shown in Fig. 6. For instance, Luzuriaga et al. and Camovi et al. stated that using the FDM method to create a sharp tip of microneedles is challenging. Luzuriaga et al. fabricated microneedles with seven different shapes using the FDM technology. Most of the robust features exceeded the print nozzle's resolution, making it difficult for the 3D printer to

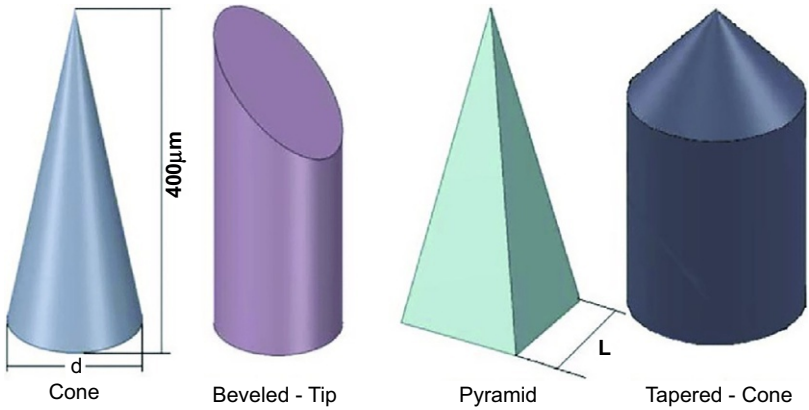

Fig. 6 Representation of different shapes of microneedles fabricated using 3D-printing technology. *(Reprinted with permission from K.T. Chang, Y.K. Shen, F.Y. Fan, Y. Lin, S.C. Kang, Optimal design and fabrication of a microneedle arrays patch, J. Manuf. Process. 54 (2020) 274–85. Copyright 2020, Elsevier.)*

replicate these designs accurately. Because the extruded layers did not adhere well to one another, several shapes could not be printed [57].

4 Evaluation of 3D-printed microneedles

4.1 Physical characterization

The physical characterization of microneedles, like dimensions, distribution, geometry, and surface morphology, can be evaluated using optical microscopy, visual inspection, and stereo microscopy. Analysis of the drop shape and measurement of the contact angle can be used to assess the surface characteristics of microneedle patches [58]. In drop shape analysis, the digital images were captured using contact angle measurement of a liquid drop on a solid surface. Then, at the position of the liquid, the tangible interface was determined by extracting the image to the drop profile [59].

The compounds included in the microneedle patches can be identified by fluorescent labeling or dyeing. Fluorescence microscopy, confocal laser scanning microscopy, or even ocular inspection can be used to see inserted molecules. Whether the molecules are present in the tip, shaft, or backing layer of a microneedle patch, visualization helps pinpoint their locations. FTIR spectroscopy can be utilized to assess coated microneedles [60].

4.2 Mechanical characterization

Mechanical characteristics of the microneedles are essential for ensuring the safety of insertion. Because of elastic and inelastic properties, the microneedles may fracture, buckle, or bend during removal or insertion. For the medication to reach the SC, it needs to have enough mechanical strength. The phrase "mechanical characterization" refers to various tests that simulate the insertion of microneedles in vivo [61].

4.3 Axial fracture force tests

In the axial fracture force test, the failure of microneedles is caused by transverse or axial loading. The test station presses the microneedle array to parallel a rigid metal surface, measuring force, and displacement while producing stress against strain curves. If microneedles fail, the force drops abruptly, and the failure force equals the highest force delivered just before the drop. The failure manner is then identified by visually inspecting microneedle arrays under a microscope and comparing them to scans made before

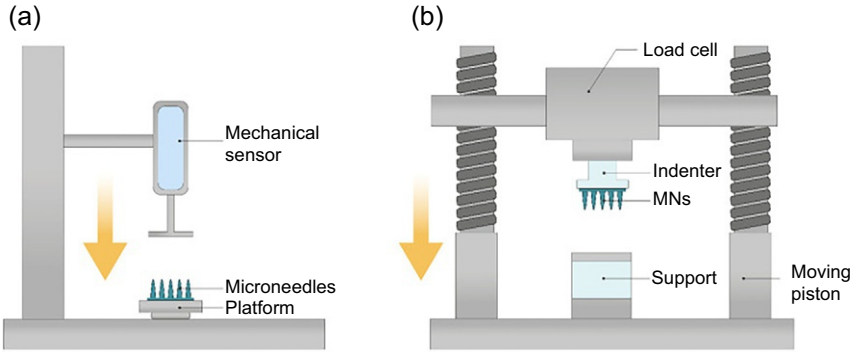

Fig. 7 Schematic representation of (A) texture analyzer for microneedle fracture analysis, (B) microneedle penetration testing [52].

the failure. The axial fracture force test uses a single microneedle array for interpretation because it cannot correlate with other microneedles from the microneedle array, as shown in Fig. 7 [62].

4.4 Transverse fracture force

Transverse fracture force tests can be done on a single microneedle and a row of microneedles. The force should be divided by the number of microneedles in the row to determine the transverse fracture force per individual microneedle. These tests are crucial for evaluating the application of microneedles. The primary drawback of this test is the need for manual alignment of the metal probe, which might result in errors in the metal probe, which has a set length and can be inaccurate [63].

4.5 Baseplate strength and flexibility tests

It is unacceptable for the baseplate to fracture during the patient's application; thus, its strength must also be established. The microneedle baseplate's flexibility ensures proper implantation on the skin's surface. If the material is flexible enough, it should be able to adapt to the skin's irregular topography without breaking. A three-point bending test can be used to determine this. Baseplates are positioned between two aluminum blocks, and a metal probe applies the force. The force necessary to break the baseplate can be calculated by looking force-distance curve's highest peak value. The baseplate's bending following a fracture can be used to determine its flexibility [63].

4.6 Insertion force tests

Due to the skin's innate viscoelasticity, microneedle insertion capacity allows the prediction of the microneedle length needed for insertion into the skin. To achieve a thorough and comparable evaluation of fracture forces, measuring the insertion force needed for the microneedle to pierce the skin is essential. The forces required to fracture the skin should be far greater than those needed to implant microneedles. Microneedles can be manually inserted or applied with a variety of applicators. Compared to manual insertion, applications offer controlled insertion conditions and reduced variability. A more comprehensive array of insertion forces can be used manually inserting [64]. The insertion force required to penetrate the stratum corneum is about 0.098 N/needles, but in some studies, only 0.03 N/needles insertion force is used to penetrate the stratum corneum [65]. After the application of microneedles into the skin, the insertion depth can be identified using histological cross-sectioning by staining of skin with different dyes like trypan blue, gentian violet, and methylene blue. These dyes help to visualize the microchannels created by microneedles in the epidermis. Only the epidermal cells are stained, and stratum corneum cells remain the same as before the insertion of microneedles. The penetration ratio of the microneedles can be calculated as the number of spots stained observed and the total number of microneedles per array [66]. The main drawback of dye staining is the overestimation of micropore diameter by the lateral diffusion of dyes. The dyes can inject into the skin through hollow microneedles [67].

OCT is a valuable technique for assessing the geometry of microneedles that affects skin penetration and in-skin dissolution. This noninvasive technique enables real-time assessment of the depth of microneedle penetration into the skin. Neither mechanical sample manipulation nor skin excision is used. It has a visible depth limit of 2000 μm [68]. If the microneedles are transparent, OCT can be used for learning about skin recovery after microneedle removal, and soluble polymeric microneedles dissolve in situ [69].

Another noninvasive technique, XRTCT, employs a series of X-ray images at various rotation angles to produce 3D volumetric data that enables 3D visualization of the insertion of the microneedles. The main drawback is that it can only detect microneedles composed of materials like gold with X-ray contrast qualities. Moreover, it is not possible to identify precisely the extent of skin penetration by the microneedles [70]. Instead of biological tissues, Larraneta et al. developed an artificial membrane constructed of

Parafilm M, a mixture of hydrocarbon wax and polyolefin. The mechanical characteristics and skin elasticity are simulated by Parafilm M, which also enables a quick and efficient evaluation of the insertion depth of various microneedles [71].

4.7 Skin irritation and recovery studies

Mild and temporary erythema may develop as a side effect after microneedle administration, depending on the size, composition, and kind of the given medication. Both stereo microscopy and dermatoscopic observation of the irritation are possible. The Draize method, which involves macroscopically observing the skin before and after applying microneedles and grading dermatological changes according to the degree of erythema and edema, can be used to measure the degree of skin irritation even though it is subjective and varied. By observing changes in the skin over time using digital photos, skin recovery can also be evaluated [72].

4.8 In vitro permeation studies

Diffusion cells can estimate the amount of drug delivered to the skin. The test is often carried out on animal skin, ex vivo humans, or a synthetic polymeric membrane between the donor and receptor compartments. The membrane diffuses the observed permeant from the donor compartment into the receptor compartment [73]. High-performance liquid chromatography can assess the transdermal delivery of the permeant from microneedles from the receptor compartment. The intradermal drug delivery can be evaluated using extraction and analysis of the compounds from the skin sample [74].

Flow-through (Bronaugh type) and Static (Franz type) are the two types of diffusion cells. Franz-type diffusion cells can be classified as upright or side-by-side cells depending on the skin orientation inside the diffusion cell. Nowadays, skin permeation studies rarely use side-by-side static diffusion cells due to the skin damage induced by completely submerging the stratum corneum and dermis in the donor solution and receptor medium while simultaneously agitating the solutions. Moreover, excessive and extended hydration of the stratum corneum through the donor compartment may result in an exaggerated penetration profile [75]. In an upright diffusion cell, skin clamping between an open donor compartment and the stratum corneum side of the cell that faces the receptor compartment contains the receptor media, simulating the environment where the skin is typically

exposed [76]. In the Franz diffusion study, maintaining the skin's elasticity is essential. If the skin elasticity loses, overpenetration of microneedles is caused in dermal tissues. To overcome this, the Franz diffusion cell's total skin thickness is used [77].

In a Bronaugh-type diffusion cell, the sink condition is maintained in a flow-through cell using a peristaltic pump, ensuring continuous receptor media flow via the receptor chamber. This can imitate dermal circulation, which carries exogenous materials from the absorption site. Moreover, these cells can sample automatically, eliminating laborious sampling time points and permitting continuous monitoring of the absorption patterns. Despite these benefits, Franz-type diffusion cells are used more frequently due to the complexity and high expense of these flow-through cells. Microneedles are often applied to the skin before the donor compartment is mounted on the receptor compartment, in contrast to the conventional procedure of in vitro Franz diffusion cell permeation tests [78].

4.9 In vivo permeation studies

Ex vivo and in vitro models cannot completely capture the complexity of in vivo skin diseases. In vivo studies are necessary to understand how medicines provided by microneedles are metabolized, eliminated, and absorbed into the body. The skin's thickness and elasticity must be considered while selecting the optimal in vivo model. Even though pig skin differs from human skin in terms of its structural, histological, and morphological characteristics, transdermal dispersion studies work best with this animal [79]. The skin is more porous than human skin, and rodents, particularly rats, are a good choice for evaluating microneedles' effectiveness because they are accessible, affordable, and easy to handle. The application site should be considered while creating in vivo models for microneedles performance evaluations. The skin's permeability and barrier function vary depending on the anatomical site. Achieving consistent outcomes also heavily depends on the thickness and rigidity of the injection site, which might be impacted by lymphatic uptake [80].

5 Combination of 3D-printing microneedles with other technologies

For the fabrication of microneedles, 3D printing technologies can be combined with other technologies like cell encapsulation, electrodes, and microfluidics.

5.1 Combination of 3D-printed microneedles with microfluidic technology

Microfluids are designed to control a small quantity of fluids in the microscopic channel. The main advantage of this technology to require a nanoliter of fluids is cost-effective, ease of usage, high specificity and sensitivity, less contamination, high analytical output, and facile parallelization via multiplexing [81]. Microfluidic devices are fabricated from glass, polymers, metal, and quartz using 3D printing, photolithography, hot embossing, and laminate. Over the decades, this device has been incorporated into medicine, the pharmaceutical field, and biologics, like screening and analysis of drugs, medical diagnostics, bioassays, and tissue engineering [82]. In recent years, microneedles combined with microfluidic technology and fabricated using 3D printing gained considerable interest because of their size. Combining these technologies has more effect on the extraction of interstitial fluid and accurate drug delivery. Stereolithography and two-photon polymerization (TPP) are the 3D-printing methods used to fabricate microneedles and microfluidic devices [83].

Yeung et al. fabricated a combination of microfluidic hollow microneedles using the SLA 3D-printing method for transdermal drug delivery. The geometrics of the hollow microneedles can determine the penetration depth of the microneedles, as shown in Fig. 8. Two of the most frequently reported 3D-printed microneedle designs were included in the printed geometries: a conical microneedle with tapered sidewalls and a pyramidal microneedle with a triangular apex and base, and fine-tip syringe-shaped microneedle was also designed. The syringe-shaped microneedle design enhanced insertion depth and ease of insertion compared to other methods studied, according to insertion trials conducted using Parafilm M. The microneedle design in the form of a syringe was therefore moved forward for additional research [84].

Rad et al. fabricated cylindrical hollow microneedle-open microfluidic channel devices that combined TPP, soft embossing, micromolding, and microfluidic technology. From the tip to the base of the microneedles, an open channel was positioned along the side of the body of the microneedles, as shown in Fig. 9. Then, this available channel was extended to a reservoir at the microneedle base. Microneedles were fabricated using Zeonor 1060R Cyclo Olefin Polymer, thermoplastic pellets, and prepared master templates made of PDMS [85]. The oxygen plasma treatment was performed to make the surface of microneedles and open microfluidic channels more

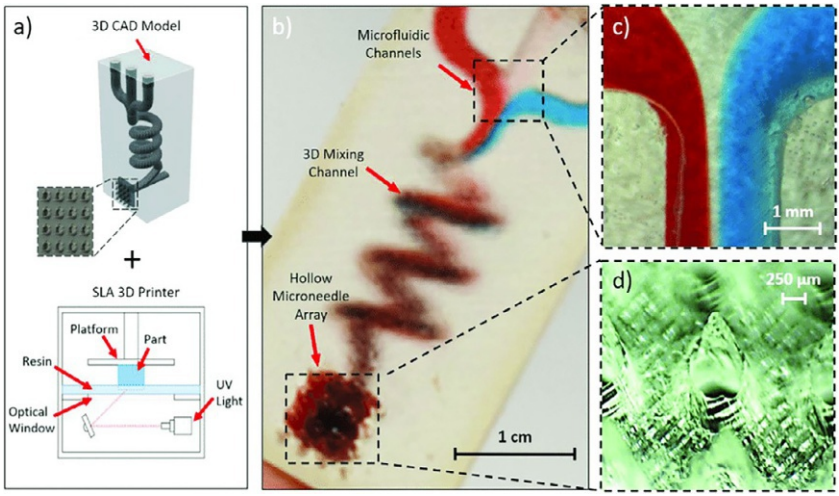

Fig. 8 (A) Schematic representation of microfluidic enabled hollow microneedle CAD design fabricated using SLA 3D printer. (B) The fabricated microneedles have three inlets converged into hollow microneedle array outlets from the 3D spiral chamber. (C) Close-up of the inlet junction visualizing the convergence of red-dyed, clear, and blue-dyed solution streams. (D) Close-up of the hollow microneedle array. *(Reprinted with permission from C. Yeung, S. Chen, B. King, H. Lin, K. King, F. Akhtar, et al. A 3D-printed microfluidic-enabled hollow microneedle architecture for transdermal drug delivery. Biomicrofluidics 13 (6) (2019) 064125. Available from: https://aip.scitation.org/doi/abs/10.1063/1.5127778. Copyright 2019, AIP Publishing.)*

hydrophilic because of the hydrophobic nature of this polymer. After conducting physical characterization tests on these unique microneedles, fluorescein delivery was examined as a model drug. Microneedles were dipped into a concentrated fluorescein aqueous solution. Two-photon optical sectioning was used to see the fluorescein's penetration of a rabbit ear. This proof-of-concept study showed how 3D microneedle prototyping, TPP, micromolding, and embossing might be used for drug delivery [86].

5.2 Combination of 3D-printed microneedles with carbon electrodes

Various biosensors have been developed to find biomarkers in sweat and saliva to continually monitor the level of metabolites (such as glucose, lactate, and uric acid) in a noninvasive manner. The biosensors measure the real-time analytes in a biological sample [87]. Numerous materials can be used to create biosensor electrodes, like gold, carbon, silver, nickel, and

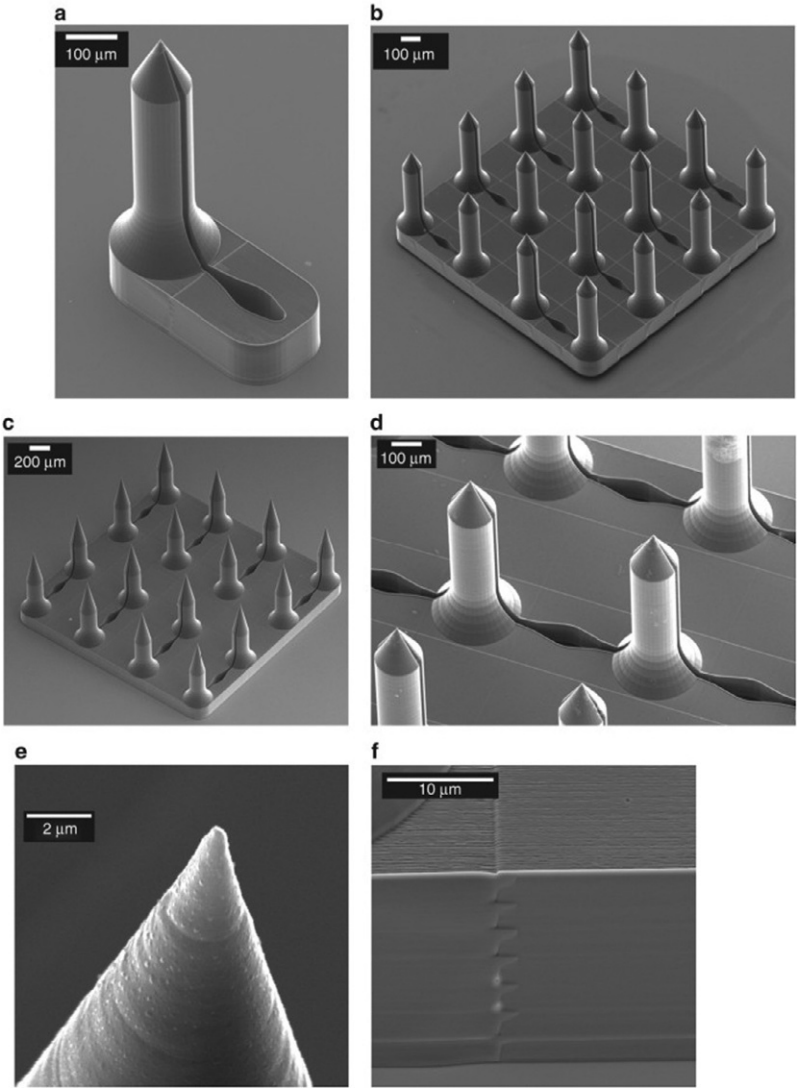

Fig. 9 (A)–(F) SEM images of open channel microneedle containing reservoir fabricated using 3D laser lithography. *(Reprinted with permission from Z.F. Rad, R.E. Nordon, C.J. Anthony, L. Bilston, P.D. Prewett, J.Y. Arns, et al. High-fidelity replication of thermoplastic microneedles with open microfluidic channels, Microsyst. Nanoeng. 3 (2017). Available from: https://pubmed.ncbi.nlm.nih.gov/31057872/. Copyright 2017, Springer Nature.)*

platinum. An electrode's material can be chosen depending on the sensor's intended use. Carbon is most frequently employed as an electrode in electrochemical biosensors because it is inert, inexpensive, biologically compatible, electrically conductive, and has a large surface area [88]. In recent years, carbon electrodes combined with microstereolithography-based 3D

printing for monitoring in situ analytes. For example, Miller et al. developed electrochemical sensing devices fabricated using microstereolithography in tetrahedron-shaped hollow microneedles and inserted carbon electrodes into the hollow microneedles. These microneedles demonstrated biocompatibility with neonatal human epidermal keratinocytes and human dermal fibroblasts, and their integrity persisted even after being removed from the skin. By using palladium-catalyzed oxidation, the carbon electrodes built within the hollow microneedles also demonstrated the ability to detect the presence of ascorbic acid and hydrogen peroxide [89].

Miller et al. fabricated a biosensor based on microneedles that can multiplex in situ pH, glucose, and lactate detection. eShell 300, an acrylate-based polymer, created a hollow MN using projection-based microstereolithography. The microneedles were then positioned on a flexible flat wire with an aperture laser-ablated and filled with a carbon paste carefully prepared to detect lactate, glucose, or pH. The instrument could be used to monitor the tumor microenvironment and specifically detect changes in pH, glucose, and lactate over physiologically relevant concentrations [90].

5.3 Combination of 3D-printed microneedles with cell microencapsulation

In regenerative medicine and drug delivery, cell microencapsulation in a biocompatible matrix has become more attractive in recent years. In this technique, cells are enclosed in a semipermeable membrane made of a biocompatible polymer, allowing the interchange of waste products, nutrients, oxygen, and medicinal substances secreted by the cells [91]. The semipermeable membrane helps in protecting against the elimination of cells by antibodies, high molecular weight molecules, and immune cells [92]. Cell microencapsulation is used for the direct delivery of cells to the desired wound area in the context of regenerative medicine. These cells will then encourage the creation of new tissue and aid in the healing of wounds by secreting growth factors and cytokines. Cell microencapsulation has been utilized with the 3D printing of MNs for drug delivery. For instance, Farias et al. evaluated the viability of delivering human hepatocellular carcinoma (HepG2) cells enclosed in crosslinked alginate capsules using hollow MNs made from biocompatible resin (FLGPCL02 photo resin), which are fabricated using SLA 3D printing [93].

6 Future prospects and conclusion

In recent years, microneedles have been an emerging biomedical device for delivering drugs through various routes. Microneedles are fast-growing devices because they are low-cost, patient-friendly, and self-administered, and deliver large molecular drugs, biomolecules, and proteins. There are various microneedles and solid microneedles in the market as derma rollers and derma pens. However, more clinical trials are needed before commercializing new studies on biodegradable microneedle patches. The design objective of these microneedle systems is to deliver a sustained release of multiple types of biomolecules in a controlled manner dependent on the biological environment's responses. Since the acceptance, safety, and immunogenicity of degradable microneedle patches are more challenging, the clinical studies of these patches provide a problem. The main drawback of polymeric microneedles is stability, accurate drug loading, and robustness.

To overcome these issues, 3D-printing technology is used to design and fabricate microneedles using various technologies. These provide accurate shape and size of the microneedles. Various microneedles fabricated using 3D print have a potential advantage in drug delivery and extraction of interstitial fluid and controlled vaccine delivery. The biosensing device and microneedles have a particular benefit in disease and insulin monitoring. The development of microneedles combined with biosensors can help with high cost-effectiveness in the future. Blood extraction through microneedles can be developed for analysis instead of the hypodermic blood drawing technique.

Therefore, future studies may be focused on the development of a fast fabrication method of microneedles using 3D-printing technology with high-resolution needles and for enhancing laser beam method for fabricating microneedles.

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CHAPTER 19

Microneedle-based electrochemical sensors for health monitoring

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1 Introduction

The rising world population, the increase in life expectancy, and the sedentary lifestyle have triggered an increment in care expenditure making healthcare an unsustainable system. Thus, the ongoing healthcare system is not bringing any hope for addressing current and new coming diseases (such as COVID-19), better patient treatments, or enhancing the lifestyle of chronic patients. For this reason, a shift in the present healthcare model needs to be addressed now for the sustainable healthcare system of the future.

In the last decade, a new paradigm has emerged bringing expectations for better healthcare, i.e., decentralized health monitoring. The decentralization of health data has been possible due to the development of portable and wearable gadgets at affordable costs, which enables massive data capture for rapid decision-making processes. In this direction, wearable devices have been governed by mainly physical sensors able to monitor heart rate, position, and/or activity, which, with proper algorithms, can facilitate the detection of cardiovascular diseases, stress, neurological damage, etc. Thus, wearable technology has brought an unprecedented way for data gathering with enormous value to be used in healthcare. However, the decentralization of biochemical analysis is still the holy grail of wearable devices to be used in the healthcare of the future.

Currently, biochemical analysis is mainly centralized in clinical laboratories with time-consuming machines and expensive reagents. To develop a sustainable healthcare system, the biochemical analysis needs to be decentralized, meaning that the biochemical information can be obtained anywhere at any time through, for example, a wearable device, without the necessity to extract a sample and send it to a central laboratory. The unique and most

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successful case of a biochemical sensor able to provide continuous health information is the continuous glucose monitoring system. The ability to monitor glucose in diabetic patients maximizes the information during daily life allowing for optimal treatments. This monitoring system proves the success of biochemical continuous monitoring and triggers the efforts of many researchers to continue in this direction.

Blood is the main biofluid utilized for biochemical analysis as it provides a circulatory and continuous flow of the biofluid over the body being in contact with all the body parts. Therefore, any alteration of the body's status can be reflected in the blood's biochemical composition. This is the reason why physicians consider biomarker levels in blood essential for diagnosis and therapeutic decisions [1]. However, blood is extracted invasively that can cause harm and discomfort. New biofluids are being used for diagnosis purposes, and new sensors are designed to be used in such minimally invasive or noninvasive biofluids [2]. These biofluids for peripheral biochemical analysis are mainly saliva, sweat, urine, tears, and dermal interstitial fluid (ISF) [3]. Despite the attractiveness of these peripheric biofluids and the easiness of extraction, they also exhibit drawbacks in terms of biofluid composition or clinical relevance (Table 1). These aspects need to be considered when developing wearable biochemical sensors for the monitoring of biomarkers. One critical feature is the correlation of the levels of the analyte with blood levels. This is essential because physicians only trust levels reported in blood for diagnosis or therapy. Furthermore, details on the correlation of biomarkers with blood for clinical purposes can be found in the literature [3]. Importantly, the size of the biomarker or analyte plays a critical role on the partitioning of the analyte toward peripheral biofluids from blood. Thus, larger biomolecules and macromolecules cannot diffuse, or at less extent, to other biofluids from blood. In contrast, ions and small molecules can diffuse and can be found in similar concentration than in blood. However, there is a lack on research in the area of correlation of biomarkers from peripheral biofluids to blood, which is needed for the further deployment of wearable biochemical sensors.

According to the table, among the most attractive biofluids for health monitoring are ISF and sweat. Sweat has gained much attention due to the noninvasiveness nature of the sample. When looking at human physiology, sweat generation is a thermoregulation process and a mechanism for the excretion of essential body molecules, which can become an informant of the health status. In addition, sweat can work as a binary indicator for exposure to exogenous chemical species [5]. On the other hand, the biochemical

Table 1 Comparison of biofluid features for health monitoring.

Biofluid	Liquid properties	Advantages	Drawbacks	Correlation with blood[a]	Challenges in sensing	Example of use case
Blood	A mixture of cells and liquid	– Clinically relevant – Constant composition	– Painful extraction – Clotting	–	– Complex matrix – Interferents from cells – Biofouling	General biochemical analysis
Saliva	Highly viscous, a mixture of debris, mucus	– Easy extraction – Painless withdrawal	– Variable secretion rate – Contamination from food intake	Diluted/except higher during drug consumption	– Variable composition (electrolytes and pH)	Drug consumption
Sweat	Clear fluid	Availability through skin	– Difficult extraction – Contamination from the skin/ evaporation	Diluted	– Variable composition (electrolytes and pH)	Sports monitoring and cystic fibrosis
Tears	Clear fluid	Clean matrix	– Discomfort in the extraction – Low amount	Diluted	– Integration of the sensor – Miniaturized device	Diabetes
Urine	Clear fluid	– High volume – Painless obtention	– Privacy issues – Residual biofluid – Delayed levels	No direct correlation/ correlation with metabolites	– Variable composition (electrolytes and pH)	Drug use
ISF	Clear to viscous fluid	– Availability through the skin – Constant composition	– Difficult extraction – Small volume	Similar[b]	– Integration of the sensor – Mechanical robustness	Diabetes

[a]It depends on the analyte of interest.
[b]For biomarkers and analytes in the low kDa range [4].

correlation between sweat and blood remains poorly understood, being mostly investigated for well-being purposes (i.e., sports practice, dehydration, and electrolyte imbalance) [6,7]. Thus, research efforts on the physiological knowledge of sweat chemistry need to be first addressed before continuing the development of sweat sensors for health monitoring. ISF is therefore the best candidate for health monitoring purposes due to its proximity to blood vessels [4,8]. Its similar correlation of biomarker/analyte levels with blood makes ISF a clinically relevant biofluid. In addition, ISF maintains some features that are essential for the biosensors' functioning such as a constant composition in terms of electrolytes and pH that aids to keep a stable signal, as well as the high availability of the sample through our entire body, i.e., skin. In addition, ISF has three times less protein content than blood [9] and no cells, which decrease the challenge of dealing with biofouling. In contrast, ISF volume is limited to about 120 $\mu L\,cm^{-2}$ in the thickest regions of the dermis (ca. 3 mm) [10] and biosensors need to disrupt the epidermis layer (i.e., outer layer of the skin) to reach the dermis layer where ISF is located. Overall, if the design of the biosensor can tackle the piercing of the skin and measurement on the dermis layer, ISF is a valuable candidate for health monitoring [4].

There are several technologies for the monitoring of biomarkers in ISF. In the last two decades, biosensors have risen as valuable tools to detect and monitor multiple parameters in biofluids [11]. In addition, the discovery of nanomaterials has broadened the applicability of biosensors and provided an enhancement in the sensitivity and the decrease of the limit of detection (LOD), which is critical for the determination of the low concentration of analytes [12–14]. Among the technologies for biosensing, colorimetric, and electrochemical sensors are the most employed, being the latter the most used readout due to its intrinsic digitalization of the information. The nature of electrochemical sensors translates the (bio)chemical interactions between the analyte and the transducer into an electrical output, which is directly used in the digital domain, maximizing the digitalization of the data [15].

The present trend in electrochemical sensors, and it can probably be extrapolated to other transduction mechanisms, is to integrate the biosensor into wearable platforms. Wearable biosensors offer advantages over regular point-of-care tests (POCT), such as seamless integration with the body, minimal user intervention, and maximal collection of data. On the other hand, several challenges are accompanied by the design of wearable devices in which stretchable and flexible materials need to be employed, mechanical resistance to deformations during daily activities of the wearer, analytically

robust biosensors, and last but not least, the miniaturization of the biosensor and the electronics. Wearable electrochemical sensors have been mainly developed for the monitoring of biomarkers in sweat [16–19] and ISF [20–23]. In addition, other matrices, such as saliva [24,25] and tears [26,27], have been used along with wearable designs, such as mouthguards and contact lenses, respectively. Wearable electrochemical sensors for health monitoring through sweat have been mainly designed as: (i) epidermal patches for monitoring electrolyte imbalance [28–30], sweat loss [31,32], metabolite monitoring [33–35], and (ii) textile-based sensors for electrolyte loss and dehydration monitoring [36–39]. Concerning the analysis of ISF, some inspiration has been obtained from commercial devices for continuous glucose monitoring, which uses ISF as the biofluid [40,41]. This technology consists of a short needle (5 mm) with an embedded electrochemical sensor that reaches the dermis layer for in situ ISF analysis [42,43]. Despite the technical advances brought by this wearable patch for glucose monitoring, the length of the needle makes the device minimally invasive and painful during its insertion. To overcome the invasiveness of such devices and maintain the advantages of continuous health monitoring in ISF, microneedle (MN) technology has been recently adopted for the development of electrochemical sensors.

Traditionally, MN technology has been used to construct MN arrays on patches for transdermal drug delivery due to its ability to disrupt the external layer of the skin and reach the dermis for more effective drug delivery in ISF [44,45]. The successful application of MN array patches (MAPs) in the drug delivery field has provided reasons in the field of bioengineering and analytical chemistry among others to employ MNs as a wearable design to develop electrochemical sensors. In this direction, MN-based electrochemical sensors (MESs) are currently a rising topic being developed toward the analysis of multiple analytes that will create an avenue of tools for the healthcare of the future. Although MESs are the predominant research in the continuous analysis of ISF, MAPs can be coupled with colorimetric readout [46,47] or used for sample extraction and ex situ analysis with a lateral-flow assay [48]. MAPs for ISF extraction are another compelling technology that can be integrated with biosensors and applied in the healthcare of the future. However, MAPs for ISF extraction are out of the scope of this chapter. Information in this area can be found in the literature [49–51].

It is worth mentioning that POCT devices have been extremely useful for the rapid diagnosis of diseases (reaching the top of success during the COVID-19 pandemic). Therefore, POCT tests, such as lateral-flow

immunoassays or the glucometer, have also facilitated the decentralization of the biochemical analysis but only at a specific time, meaning that relevant events can be missed in comparison with continuous monitoring systems. Both technologies are valuable for the healthcare system of the future, but continuous monitoring can be more relevant than POCT as it provides constant digitalization of the information to enable personalized, predictive, and lastly preventive healthcare. Due to the importance of health monitoring, the following sections will be detailing MES for continuous monitoring of health parameters in ISF.

2 Microneedle-based electrochemical sensors

2.1 Types of MN arrays and construction of MN-based electrochemical sensors

The engineering of a MES consists mainly of (i) the fabrication of a MAP; (ii) the modification of the MAP with conductive materials to develop a set of electrodes that becomes the electrochemical cell; (iii) the functionalization of the working electrode (WE) with a recognition element, membrane or nanomaterial; and (iv) integration with the connector and electronics (i.e., potentiostat with wireless transmission). Moreover, the development of a user-friendly cell phone application to display the results is necessary for daily use in a real scenario, but it is here excluded as an essential element for the construction of MES.

The morphology of the MN is an essential aspect to consider when designing electrochemical sensors (Fig. 1). Several aspects are common in the design of MNs: (i) Tip sharpness is crucial for easy and painless penetration of the MN into the skin; (ii) the width governs the insertion in the skin

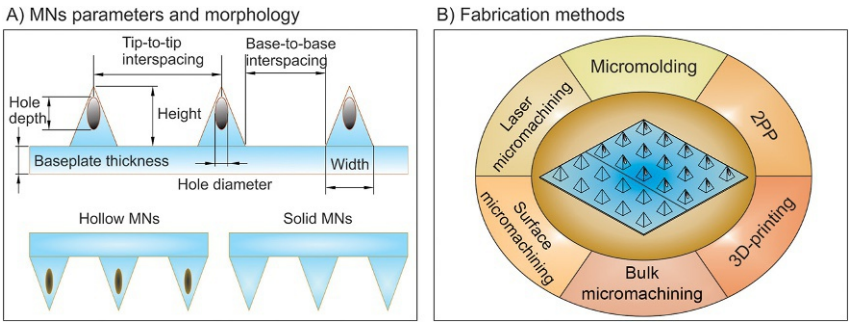

Fig. 1 Morphologies, parameters, and fabrication methods of microneedle array patches: (A) MNs shapes and parameters, and (B) common fabrication methods.

by providing sharper MN; and (iii) the base-to-base interspacing is related to the force needed to insert the MAP, meaning that with less density of MNs, easier the insertion. The shape of the MN depends on the sensing strategy employed. Among the most used: (i) solid MNs permit easy manufacturing reaching a high resolution at the tip and allowing a high surface area in contact with the ISF (an important parameter to design high electrocatalytic surface area and sensitive sensors); and (ii) hollow MNs, which can be used for ISF extraction and to turn into sensors by filling the holes with conductive paste, embed micron-sized wires or an entire electrochemical sensor.

Multiple fabrication methods have been used to manufacture MAPs for electrochemical sensing [52,53]. Among the methodologies, a wide range of features can be selected to be used depending on the requirements for MAP production (Table 2). Micromolding is one of the most used methods for MAPs in drug delivery, which can be also used for MES. It consists of the design of a template (the mold) where the solution of interests is cast to be later unmolded to obtain the desired MAP. This method offers high versatility in the design of solid MAPs as the materials can be tuned and modified according to the application. Mainly, polymeric materials dissolved in a solvent are used [54,55]. Two-photon polymerization (2PP) has recently attracted much attention due to the high resolution offering the possibility to design nanometer range features [56]. In contrast, this technique is expensive due to low production rates, long printing times, and equipment costs. An increasing number of materials are being employed in 2PP, such as acrylate-based polymers, water-soluble materials, or polyethylene glycol

Table 2 Comparison of fabrication methods for MNs array patches.

Fabrication method	Resolution/ range	Template	Time	Materials	Scalability	Cost
Micromolding	μm	Yes	Fast	High variety (polymers)	High	Low
2PP	nm	No	Slow	Low variety (resins)	Low	High
3D printing	μm	No	Fast	Low variety (resins)	Mid	Mid
Bulk micromachining	nm	Yes	Slow	Restricted (silicon)	Mid	High
Surface micromachining	nm	Yes	Slow	Low variety	Mid	High
Laser micromachining	μm-nm	No	Fast	High variety	Low	High

[57]. Another additive manufacturing technique that reached commercial maturity is 3D printing [58,59]. Several technologies have been commercialized in 3D printing. For example, stereolithography is a cost-effective technique for reaching an appropriate resolution for MAP fabrication [60,61]. 3D printing offers high versatility in designing MAP at a low-cost, which is suitable to boost innovation in the laboratory. Micromachining has been historically used to reach micrometer and nanometer range objects. Thus, it has been widely exploited for MAP production. Bulk and surface micromachining has enabled the fabrication of MAP [62]. However, cleanroom facilities and multiple steps restrict MAP fabrication. In contrast, laser micromachining by ablation of material offers a direct writing manner to develop MAPs [63]. Still, micromachining-related techniques are expensive to allow suitable scalability of MN-based devices.

Once the MAP is manufactured and before the modification toward an electrochemical sensor, a morphological characterization of the MAP needs to be performed. Optical microscopy or scanning electron microscopy can be employed to measure the dimensions of the MAP. Subsequently, statistical analysis from the dimensions of the CAD is carried out to evaluate the accuracy of the manufacturing process. As the main goal of a MAP is the painless disruption and insertion into the skin, the piercing capability of the MAP is assessed by the parafilm test (as a skin model for penetration tests). This test allows calculating the penetration depth by counting the holes in each of the Parafilm layers [61,63]. Moreover, porcine skin is usually used to assess the ease of insertion in the skin by counting the holes created. Both experiments can be performed by the application of thumb pressure or by using a universal testing system to vertically apply a controlled force. The universal testing system can be also used to test the resilience of the MAP by applying increasing force until the deformation of the MAP. This test is useful to know the maximum force needed to completely insert the MAP into the skin and, accordingly, to design MAP applicators.

2.2 Electrochemical techniques used in MESs

Electrochemical sensors have been widely used for the detection and continuous monitoring of relevant health parameters due to their cost-effectiveness, as well as allowing the direct digitalization of the (bio)chemical data. Intrinsically, electrochemical sensors are transducers of (bio)chemical information through electrochemical events occurring at the surface of an

electrode to electrical signals that are used to measure and quantify analytes. Therefore, electrochemical sensors are ideal for this new digital era.

In MES, there are static (current = 0) and dynamic (current ≠ 0) electrochemical techniques utilized for analytical purposes (Fig. 2A) [20,21,64]. Concerning the static techniques, potentiometry is a widely used method in MES thanks to the advantage of having a simple electrochemical setup involving only a WE and a reference electrode (RE). In addition, potentiometry is a low-power consumption technique that allows the detection of specific analytes through mainly solid-state ion-selective electrodes (SC-ISEs) [28,65]. Moreover, other analytes, such as hydrogen peroxide [66] and (indirectly through hydrogen peroxide) glucose, have been reported using redox-sensitive materials, such as platinum electrodes [67]. Potentiometry measures the change in voltage between a RE and a WE when the analyte of interests interacts with a plasticized polymeric membrane, i.e., ion-selective membrane (ISM) on the WE changing the electrochemical potential (phase-boundary potential) of the electrochemical cell [68]. Potentiometry thus allows monitoring cations and anions through SC-ISEs consisting of: (i) an electrode material, (ii) an ion-to-electron transducer (normally nanomaterial with high capacitance for electric double-layer capacitance mechanism or conducting polymer for the redox capacitance mechanism), and (iii) an ISM (comprising a structural polymer, a plasticizer to confer flexibility to the membrane, an ion exchanger responsible of the ion partitioning at the phase boundary allowing the sensing principle, and an ionophore to favor the exchange of the primary ion and thus grant selectivity to the system) [69,70]. Interestingly, potentiometric ion sensors provide information on the activity of the ion, which is proportional to the concentration of the ion in the solution. The activity is a measure of the effective concentration of a species in a mixture and depends on the activity coefficient, which is calculated according to the concentration of other species found in the solution. Another important aspect in potentiometric sensors is the attainment of a suitable RE that keeps a constant potential independently of the chloride activity in solution. A typical solid-state RE used in wearable designs is based on a silver/silver chloride (Ag/AgCl) electrode covered with a sodium chloride-saturated polyvinyl butyral membrane [39,61,71]. In addition, other REs based on polymeric membranes and ionic liquids have been reported [28].

Dynamic electrochemical techniques involving redox processes in MES encompass mainly controlled-potential techniques, such as voltammetry and chronoamperometry (Fig. 2B and C). For this reason, a three-electrode

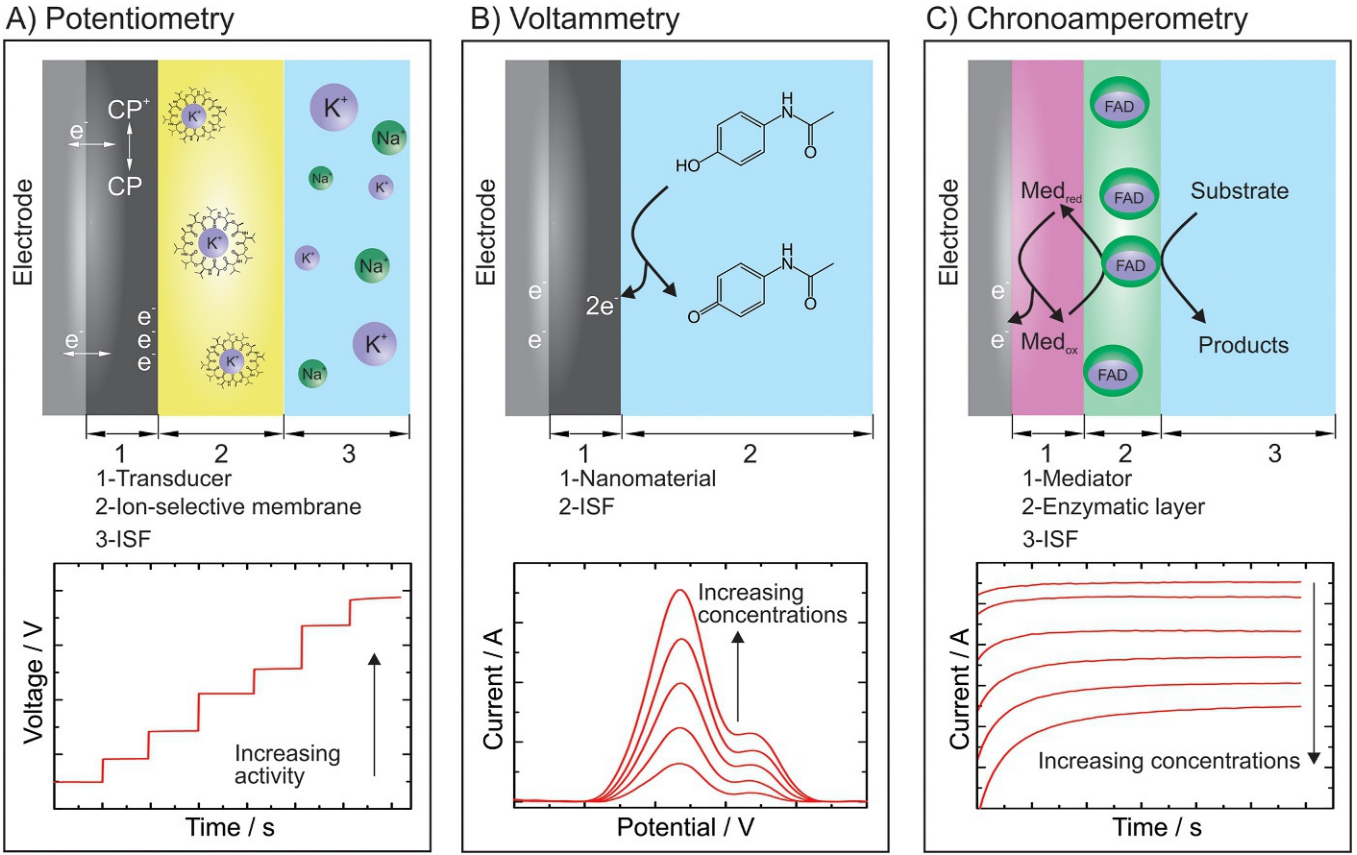

Fig. 2 Electrochemical techniques and associated electrochemical strategies for continuous monitoring of analytes: (A) potentiometry for the monitoring of ionic species based on an ion-selective membrane deposited on a MN-based electrode. The potential readout has a logarithmic relationship with the activity of the ion. Transducer is based on a conducting polymer (CP) or nanomaterials; (B) voltammetry for the direct detection of electroactive molecules by losing or donating electrons at the surface of the MN-based electrode when a redox potential is applied. The oxidation or reduction peak current height is directly proportional to the concentration of the target analyte; meanwhile, the peak potential determines the analyte; and (C) chronoamperometry used in enzymatic biosensors, and glucose is oxidized to gluconic acid and detected by oxidation of the mediator in a second-generation enzymatic sensor.

configuration electrochemical cell is needed (i.e., RE, WE, and counter-electrode (CE)). Voltammetric techniques can be divided into different methods depending on the potential excitation signal. In this way, differential-pulse, staircase, and square-wave excitation signals lead to differential-pulse voltammetry (DPV), cyclic voltammetry (CV), and square-wave voltammetry (SWV), respectively, as the most used electroanalytical methods in MN-based voltammetric sensors. Voltammetric techniques are employed for the direct analysis of electrochemically active targets, meaning that the analytes can be directly oxidized or reduced at the surface of the electrode by applying a specific voltage to the electrochemical cell. In this case, the redox potential will depend on the target molecule, electrode's material, and chemical composition of the solution (e.g., pH and electrolyte). Voltammetry is utilized for the detection of small molecules, such as ascorbic acid, uric acid, or drugs (Fig. 2B). Moreover, in situ derivatization strategies have been coupled on the WE to convert nonelectroactive to electroactive molecules, which can subsequently be detected [72,73]. To increase the sensitivity and LOD, a huge variety of nanomaterials and hybrid materials have been widely used [74,75].

Chronoamperometry is currently the most employed technique as it is used by commercial glucose monitoring systems. It consists of the application of a constant electric potential through time to the WE resulting in the generation of current from faradic processes between the WE and CE. The amount of current generated is correlated with the amount of analyte found in the biofluid (Fig. 2C). Enzymatic biosensors run on chronoamperometry providing a repeatable signal and appropriate LOD for applications in healthcare [76]. Principally, three generations of enzymatic biosensors have been described [77]. The first generation employs the product (i.e., hydrogen peroxide) of the enzymatic reaction (from oxidase-type enzymes) to trigger the redox process at the surface of the electrode leading to the faradaic current. To decrease the overpotential applied to trigger the reduction or oxidation of hydrogen peroxide, a mediator is normally introduced on the electrode's surface. Afterward, a low electrical potential is applied avoiding issues with redox active molecules in the biofluid and decreasing the power consumption. The second generation involves the oxidation of the cofactor that enables the enzymatic reaction. Some enzymes have the cofactor in their structure (flavin adenine dinucleotide in glucose oxidase), while other enzymes need an external cofactor such as nicotinamide adenine dinucleotide (NAD) to be utilized in the enzymatic reaction. The cofactor is oxidized by reducing a mediator (e.g., ferrocene or tetrathiafulvalene),

which is subsequently oxidized at the electrode's surface by applying a constant potential generating an output current. This redox cycle enables highly sensitive detection of the analyte (i.e., substrate of the enzymatic reaction). Last but not least, the third generation facilitates the direct reduction of the cofactor at the electrode's surface by using engineered enzymes. To obtain proper sensitivity, the enzymes (and particularly the cofactor) need to be properly oriented, attached, or bonded to nanostructured electrodes (close distance of the redox center to the electrode's surface) to facilitate the electron transfer [78].

2.3 Modification of the MAP toward a functional MES

Two main strategies can be designed to translate a regular MAP into a MES:

(i) The use of hollow MNs to extract ISF and in situ perform the electrochemical detection at the backside of the MAP [63]. This strategy does not involve the use of conductive materials on the MAP. However, low extraction volumes, microfluidic designs, and integration with miniaturized electrochemical sensors are needed leading to complex systems. In this strategy, the MAP can be attached or coupled to the electrochemical sensor such as screen-printed electrodes. MAPs can also be composed of solid swellable MNs that can uptake ISF and bring it into contact with the electrochemical sensor [79] or porous MNs that can get hydrated toward the electrodes [80].

(ii) The modification of the MAP with conductive materials to attain the MES can perform in situ electrochemical analysis in the skin, while the MAP is pierced. After the manufacturing of the MAP, the modification with conductive materials is needed to attain two or three different MN-based electrodes (depending on potentiometric sensors with a two-electrode cell or voltammetric and amperometric sensors with a three-electrode cell). This step will vary according to the type of MAP. Solid MAP can be modified by the sputtering of metals on top of the MAP [81,82] or by coating the MNs with conductive inks [71]. In addition, conductive solid MAPs can be created during the manufacturing of the MAP, e.g., by including nanomaterials in the polymeric solution used during the micromolding technique [83]. Hollow MNs have been widely used by the modification of the holes with conducting pastes [61], by introducing wire-based electrodes inside the

holes [84] or even an entire miniaturized electrochemical sensor [85]. When using MES based on conducive materials, the most attractive strategy is the employment of hollow MNs because the MN core can act as a shell protecting the electrode. Therefore, the force exerted during the penetration of the skin is less prone to damage the MN-based electrode than when a solid MN is completely modified and exposed to the skin insertion. As the analytical response of voltammetric sensors depends on the electroactive surface area in contact with the solution, solid MESs need to be completely pierced on the skin to obtain reliable results. This can also be a limitation depending on the skin thickness from person to person. In contrast, hollow MNs have the electrode's area close to the tip of the MN, which assures to reach and completely cover the electroactive surface area with ISF in the dermis layer. From the practical point of view, a hollow MAP is interesting as it can integrate two or three electrodes necessary for the electrochemical sensor by just filling certain hollow MNs with the appropriate conductive paste (e.g., silver/silver chloride for the pseudoreference electrode and carbon for WE/CE). In contrast, two or three solid MAPs are necessary to build the electrochemical cell adding complexity to the electrochemical configuration.

To confer selectivity to the WE, the MN-based biosensors can be modified with recognition elements. Naturally occurring or genetically engineered protein receptors have been widely used (i.e., mainly enzymes and antibodies) [86]. Enzymatic biosensors allow the selective detection of small molecules, and antibodies enable the recognition of a wide range of molecules from small molecules to macromolecules (i.e., other proteins). Moreover, nucleic acid receptors based on single-strand DNA and aptamers have been recently reported [87,88] as a new emerging MN type of sensor. These elements allow the binding to other nucleic acid biomarkers or therapeutic drugs. The label of these macromolecules with electroactive molecules such as methylene blue permits the electrochemical detection of biomarkers. A conformational change occurs during binding, resulting on a change in the current, which is correlated with the analyte concentration. Synthetic receptors, such as molecularly imprinted polymers (MIPs), are predetermined polymers that have been processed leaving cavities for the analyte, thus enhancing the selectivity of the sensor. There are several biosensing approaches in MIPs: (i) the electrochemical signal decreases upon binding

of the analyte to the MIPs when using a redox reporter; (ii) the impedance of the electrode changes according to the analyte binding; and (iii) the MIPs can be designed with internal redox reporters that change conformation, as well as their electron transfer properties (similar to the aptamer approach). MIPs have been employed in regular electrochemical sensors and in epidermal patches. However, there is almost no literature reporting MIPs in MNs, being limited by only MIPs for cortisol detection [89] and cytokine IL-6 [90].

Once the MAP is modified with conductive materials to become an electrode, the functionalization of the WE is essential for the successful development of the electrochemical sensor. The functionalization depends on the biosensing strategy and the recognition element employed. For example, potentiometric ion sensors are mainly limited to membrane-based functionalization. Regular functionalization approaches of the electrode's surface involve: (i) membrane coating in SC-ISEs or physical entrapment of enzymes by mixing with a membrane or covering on top of an enzymatic layer; (ii) anchoring the receptor at the surface of the electrode, for example, by the thiol bond on gold surfaces, a commonly used strategy in aptamer sensing; (iii) electrostatic interaction between the surface and the material; or (iv) electropolymerization of the material (e.g., mediator or conducting polymer), which can entrap the recognition element.

2.4 Analytical characterization of MESs

The analytical characterization of the MES is an imperative step to assess the reliability of monitoring systems. After the fabrication and modification of the MAP, a thoroughly analytical evaluation of the sensor performance at different levels is needed to truly assess its usefulness in future real scenarios. Hence, the sensor needs to prove its reliability (i) in vitro; (ii) ex vivo; and (iii) in vivo. Before starting the analytical characterization, the sensing mechanism needs to be studied by regular electrochemical experiments (e.g., cyclic voltammetry). For example, the study of mass transport, adsorption behavior of the analyte, pH influence, etc., will vary depending on the type of sensor (e.g., enzymatic sensor, voltammetric, and potentiometric). Subsequently, an in vitro analytical characterization of the MES is performed in phosphate buffer saline (PBS) solution at pH 7.4 to emulate ISF pH and electrolyte conditions (Fig. 3). In this first level, a calibration curve is executed

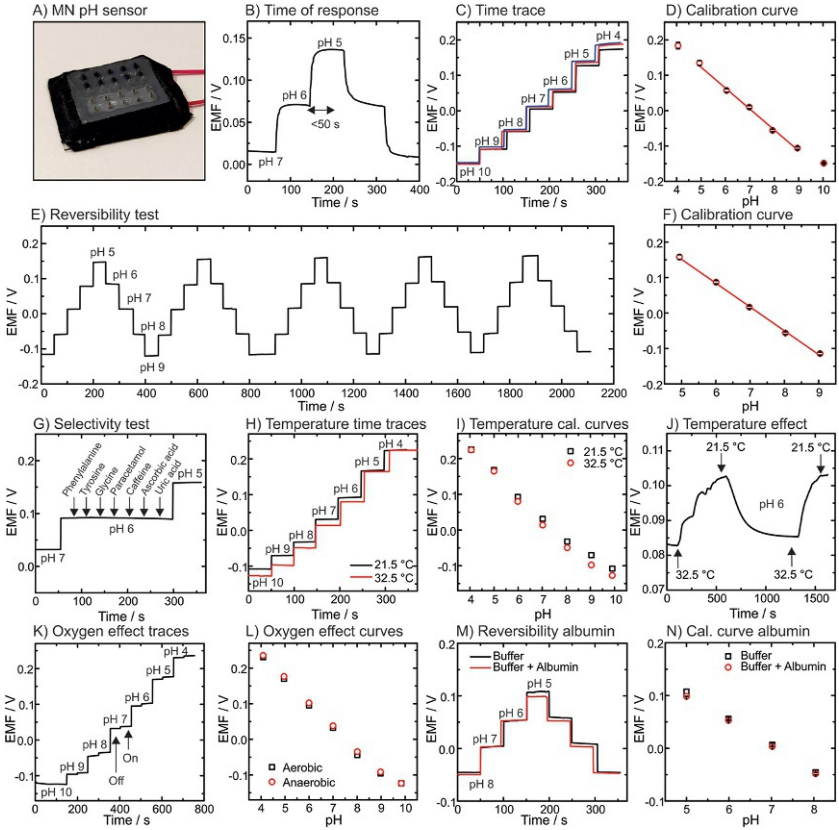

Fig. 3 Figures of merit used for the in vitro analytical characterization of MN-based electrochemical sensors. *(Reproduced with permission from M. Parrilla, A. Vanhooydonck, M. Johns, R. Watts, K. De Wael, 3D-printed microneedle-based potentiometric sensor for pH monitoring in skin interstitial fluid, Sens. Actuators B Chem. 378 (2023) 133159, https://doi.org/10.1016/j.snb.2022.133159.)*

by adding increasing concentrations of the target analyte to calculate the sensitivity or slope, the linearity, the LOD, and the limit of quantification (LOQ) of the sensor. The experiment can be done by dropping the solution on the sensor or by immersing the MN sensor in the solution. A repeatability and reproducibility study can be carried out by repeating measurements at the same or varying concentrations using the same sensor or by changing the MES, respectively. This can be performed at different concentrations within the linear range. Ideally, the linear range needs to fit within the levels of

interest of the analyte in ISF to meet the clinical application (e.g., diagnostic or monitoring purposes). Subsequently, an interferent study is performed by adding common biomolecules (electroactive) in the solution at the same levels as are encountered in ISF. This experiment can be done by adding the interferents alone or mixed at a certain concentration of the analyte. Once the linear range is determined, a reversibility study or carryover test is addressed by changing the concentration of the target upon increasing and decreasing concentrations within the clinical range [63]. This step is essential to evaluate the ability of the sensor to monitor fluctuations, one of the most valuable features of sensors for continuous monitoring. Mid-term (1 day) and long-term (> a day) stabilities are important to assess the performance for continuous monitoring while maintaining the sensor at a fixed concentration for a certain time. Skin temperature is different from the ambient temperature in the laboratory. Therefore, the effect of temperature on the calibration curve needs to be assessed. In addition, oxygen influence is worth evaluating using ambient oxygen and degassed conditions as oxygen in ISF might differ from ambient conditions. If any component of the sensor is light-sensitive, an evaluation of the influence of light should be also performed. Last but not least, biofouling can alter the analytical performance of the sensor. Biofouling is the passivation by adsorption of biomolecules that are present in the ISF on the surface of the electrode. This adsorption can block the target analyte to reach the electrode's surface, thus decreasing the electrochemical signal. Mainly, proteins and lipids can be adsorbed on the electrode's surface. Several strategies have been established to avoid biofouling [91], assuring mid- to long-term measurements: (i) nanoengineered surfaces; (ii) hydrogels; (iii) self-assembled monolayers and grafted coatings; and (iv) membranes. Considering that the sensor aims to be used in ISF, testing the analytical performance with protein-enriched PBS (e.g., using bovine serum albumin) is necessary to evaluate biofouling. If then a biofouling strategy is applied to the MN-based sensor, a full in vitro analytical characterization would be necessary to assess sensitivity, LOD, reproducibility, repeatability, reversibility, selectivity, mid-term and long-term stability, and the effect of temperature, light, and oxygen.

The next step during in vitro test is the use of phantom gels (i.e., hydrogel) to mimic the analytical performance using a solid-state electrolyte. In addition, a soaked sponge can also be used to emulate the dermis layer.

In this step, the ability of the sensor to detect the analyte is evaluated by piercing the phantom gel or sponge. Before the experiment, gels soaked or prepared with known concentrations of the analyte are employed. Thereafter, the MES is pierced into the gel and the analytical response is recorded. Ideally, a calibration curve and reversibility test are necessary to evaluate the capability of the sensor to monitor in solid state.

The second level in the full analytical characterization of the MES is ex vivo testing (Fig. 4). The ex vivo test employs usually porcine skin as a model to mimic human skin. Therefore, full-thickness porcine skin 1–3 mm is utilized. During the insertion of the MN sensor in the skin, a force needs to be exerted to disrupt the stratum corneum, go through the

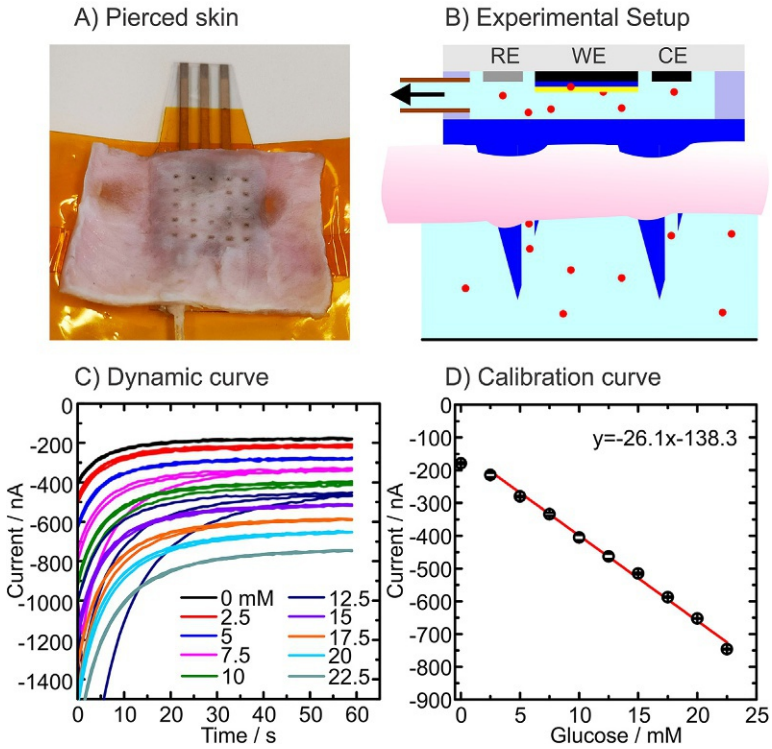

Fig. 4 Figures of merit for the ex vivo evaluation of the analytical performance of MN-based electrochemical sensors. *(Reproduced with permission from M. Parrilla, U. Detamornrat, J. Domínguez-Robles, R.F. Donnelly, K. De Wael, Wearable hollow microneedle sensing patches for the transdermal electrochemical monitoring of glucose, Talanta 249 (2022) 123695, https://doi.org/10.1016/j.talanta.2022.123695.)*

epidermis, and reach the dermis. This friction can damage the surface of the electrode and thus alter the analytical performance. To evaluate the insertion effect in the skin, a calibration curve before and after poking the MN sensor in the skin can be executed [61]. Both calibration curves are carried out in a buffer solution. Ideally, the results should indicate an identical calibration curve resulting in no damage to the sensor. To further assess the robustness, the insertion can be performed several times [71]. The second step in the ex vivo characterization is the evaluation of the ability for continuous monitoring, while the MN sensor is inserted into the skin. This ex vivo experiment is essential to emulate a real scenario by using a skin model and avoid animal testing. Thus, this experiment assures an excellent analytical performance of the MES before in vivo testing. For this purpose, the setup of the skin model consists of using a Franz diffusion cell with porcine skin. Franz diffusion cells have been widely used in drug delivery systems but less exploited in sensing systems [45,92]. A Franz diffusion cell consists of a receptor cell with the solution at a certain concentration covered with the porcine skin and a donor compartment where the MES is placed. The MNs pierce the skin, which is in direct contact with the solution and thus allowing the analyte to diffuse to the electrode. Normally, the Franz diffusion cell has a water jacket to keep the solution in the inner compartment at skin temperature. The cell has a sampling port that is used to spike the sample and allow the execution of a calibration curve. The time of diffusion of the analyte through the skin and the skin thickness (e.g., using dermatomed skin) is parameter to evaluate during the ex vivo test. Subsequently, calibration curves and stability tests while the MN sensor is inserted can evaluate the reliability of the sensor in a future scenario. This skin model enables ex vivo testing simulating the physiological conditions of an in vivo test.

The third level comprises the proof of concept of the MES during in vivo tests and the validation of the MES (Fig. 5). The proof of concept is usually performed by piercing the MES into a laboratory animal (e.g., rat, mouse, or rabbit) or directly to a human subject. The analytical readout obtained during the in vivo test (i.e. raw data) is transformed into concentration by employing a calibration curve from previous in vitro or ex vivo experiments depending on the robustness of the MES. External induction of stress or administration of certain compounds can be performed to change the levels of endogenous biomarkers (e.g., glucose or insulin) and evaluate the ability of the MES to monitor fluctuations. When dealing with the monitoring of exogenous biomarkers, the administration of the analyte of interest to the

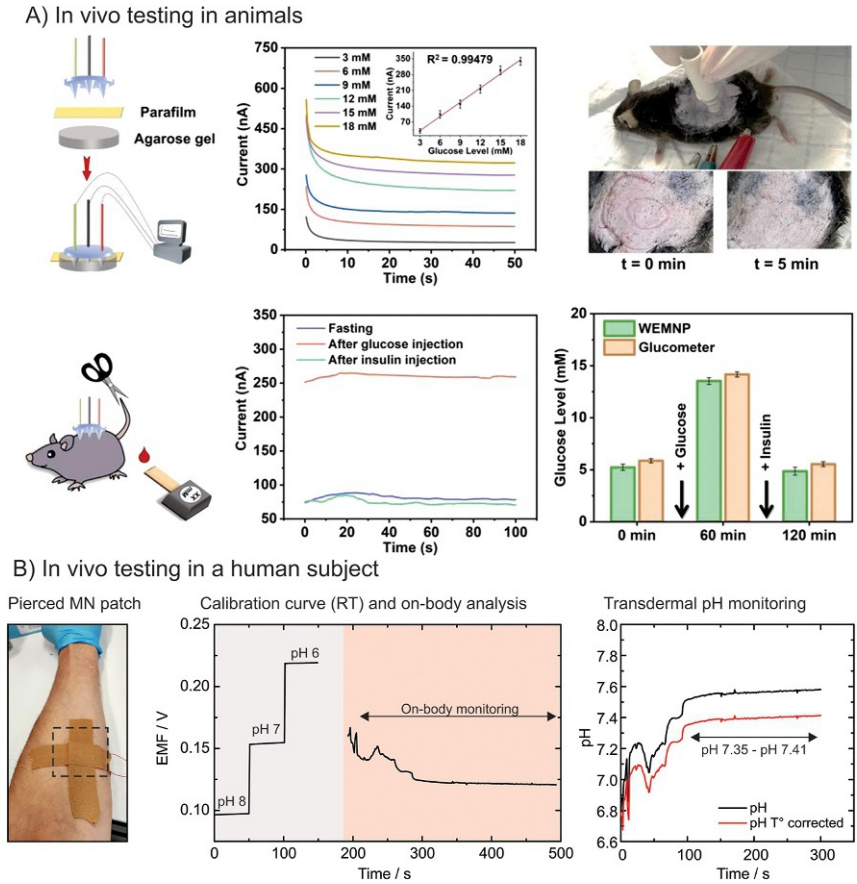

Fig. 5 Examples of in vivo testing of the analytical performance of the MN-based electrochemical sensor. (A) In vivo test in mice for glucose monitoring. (B) In vivo test in the forearm of a human subject for pH monitoring. *(Reproduced with permission from (A) L. Zheng, D. Zhu, W. Wang, J. Liu, S.T.G. Thng, P. Chen, A silk-microneedle patch to detect glucose in the interstitial fluid of skin or plant tissue, Sens. Actuators B Chem. 372 (2022) 132626, https://doi.org/10.1016/j.snb.2022.132626. (B) M. Parrilla, A. Vanhooydonck, M. Johns, R. Watts, K. De Wael, 3D-printed microneedle-based potentiometric sensor for pH monitoring in skin interstitial fluid, Sens. Actuators B Chem. Sensors Actuators B. Chem. 378 (2023) 133159, https://doi.org/10.1016/j.snb.2022.133159.)*

animal is performed. However, these experiments do not assure that the analytical readout matches the true concentration provided by the calibration curve. Several factors can influence the readout: (i) a deviation of the output can be obtained due to the lack of performance of the MES, and/or (ii) the target analyte (e.g., a therapeutic drug) can interact with the body (e.g., binding with protein), thus making less free analyte available for the

electrochemical reaction. Therefore, a validation of the MES is needed. This step can be carried out by the extraction of ISF in parallel (e.g., by blister suction device) and analyzing the composition with a standard method. It is important to consider that the validation of MN-based devices is challenging due to the lack of standardization for ISF extraction and analysis. Next, a proper comparison of the readout can be performed between MES and the standard method leading to a validated MES. Once the validation indicates a minor deviation from the standard method, the MES is reliable and ready for further trials in human applications (i.e., clinical trials).

2.5 Evaluation of biocompatibility and toxicity

MN-based technology is an interface between the body (i.e., skin) and electronics. Hence, the assurance of biocompatible and nontoxic materials is mandatory to develop a successful MES. Bioelectronic devices can display poor biocompatibility since the materials are recognized as foreign bodies by the immune system [93]. Thus, the materials used in the MES cannot be toxic and reactive to avoid inducing an undesirable immune response and inflammation that can: (i) alter the biomarker or analyte levels, and (ii) be harmful to the health of the wearer. To comply with ISO standards for the potential commercialization of MN-based devices, ISO 10993-5:2009 describes the test methods to assess the in vitro cytotoxicity of medical devices.

In MAP, the sharpness and smoothness of the MN are essential to provide a clean insertion to avoid any inflammation event after the MES application. Moreover, the materials need to be biocompatible and nontoxic. The toxicity can be tested in vitro by bringing in contact the MNs with epidermal cell lines (e.g., fibroblasts). After incubation for more than 2 days, a live and dead assay can be performed on the cells to evaluate the toxicity [94]. Sometimes, materials do not induce cell death but inhibit proliferation. Therefore, cell counting (e.g., CCK8 assay) upon increasing time in cell cultivation with the material can show inhibition. In addition, the staining of the cells with biomarkers responsible for cell proliferation (e.g., KI-67) can demonstrate proliferation inhibition [71]. The biocompatibility and toxicity of the materials for the construction of MES can be evaluated by [85,95]: (i) viability and time-course tests from direct contact of the material with the cells; (ii) viability tests caused by potential leaching of some compounds by depositing the material in a transwell and allowing the leached material to diffuse in the cell culture media, or (iii) by let the

cells growing around the deposited material. In this way, the toxicity and biocompatibility of ISMs, a key compound on MN-based potentiometric ion sensors, were successfully evaluated [95]. In addition, the employment of new materials and nanomaterials in MES needs to be constantly evaluated by proper cytotoxicity tests.

3 Application in health monitoring

Wearable MES technology has principally been applied in the detection and monitoring of analytes that are relevant to elucidate the health status of the wearer. MES technology enables the decentralization of biochemical information by bringing tools that can be employed in telemedicine, home care, and digital health. The digitized information can be subsequently employed for rapid decision-making processes by trained personnel (i.e., physicians, coaches) or artificial intelligence (AI). Such processes involve (i) diagnosis of medical conditions or diseases with the following selection of the treatment; (ii) monitoring of a health condition related to a diagnosed disease in patients with optimal treatment (e.g., diabetic patient monitoring glucose and follow-up with insulin treatment); (iii) monitoring of physiological status in well-being for a better training and understanding of the limits of the body (e.g., lactate monitoring in athletes during high-intensity sports practice); (iv) therapeutic drug monitoring (TDM) for effective treatment in disorders with narrow therapeutic range (e.g. antibiotics); and (v) overdose detection and monitoring after rescue with an antagonist by first responders.

Relevant data for health management can be provided by endogenous biomarkers or by exogenous targets. Endogenous biomarkers are biomolecules that are produced by the body. These biomarkers range from electrolytes, such as potassium or sodium, small molecules such as metabolites, nucleic acids such as DNA and RNA, to macromolecules such as proteins. Hence, an alteration of the physiological levels of these biomarkers can lead to the diagnosis of a disease or the monitoring of a health condition. Exogenous targets comprise molecules that are produced by an external source (e.g., DNA, RNA, or proteins delivered by viruses or bacteria) during an infection or administrated drugs during therapeutic treatments. Overall, the MES allows the detection and monitoring of irregular events in ISF that can be linked to health conditions and treatments.

3.1 Endogenous biomarkers

3.1.1 Electrolytes

Ions are an important indicator of the health status of the body. Ions are regular biomarkers analyzed in mostly all blood tests. Therefore, the monitoring of ions in ISF is a valuable tool to track the body's status. Generally, potentiometry has been used as the electrochemical technique in MES. A MES for the monitoring of potassium has been reported by coating solid MNs with conductive inks and a potassium-selective membrane (Fig. 6A) [71]. The beauty of potentiometric sensors is that the sensitivity does not depend on the electroactive surface area, and thus, micron-size electrodes should exhibit the same Nernstian sensitivity (i.e. 59.2 mV decade^{-1} at 25°C) as macroelectrodes. In this way, a wearable MAP with only two MNs, one as a WE and another as RE, can be used as a single sensor. pH is also a widely utilized biomarker that reflects the acid-base balance of the biofluid, which can be directly correlated with many metabolic processes of the body. Biological processes are optimal at pH 7.4, and thus, an alteration in the pH can produce dramatic consequences. pH changes can be attributed to infections, heart or kidney diseases, or local increase of lactic acid during sports practice, among others. Hence, a MES for pH monitoring is important to monitor the body's health. Several MESs for pH monitoring have been reported using different MAPs. For example, the use of a high-density polymeric MAP manufactured by micromolding and modified with gold sputtering and polyaniline to devise the pH MES [96]. A 3D-printed hollow MAP has also been engineered. The electrodes are made by filling the holes with graphite and Ag/AgCl paste with the following electrodeposition of PANI and PVB membrane, respectively [61]. Interestingly, a MES based on an array of pH sensors was used for mapping peripheral artery disease [97]. In general, PANI is a conducting polymer that can be easily electrodeposited showing excellent analytical performance toward pH and biocompatibility [98].

3.1.2 Metabolism-related molecules

Glucose is the most analyzed molecule in the world due to its beneficial application in diabetes management. Hence, numerous MES for glucose monitoring have been recently developed. Many strategies have been used to monitor glucose being the enzymatic approach the most reported. In MES, several solid MAPs have been modified with enzymatic and mediator layers [99–106]. Interestingly, a fully integrated MN-based device, including the MES and miniaturized electronics for the monitoring of glucose, lactate,

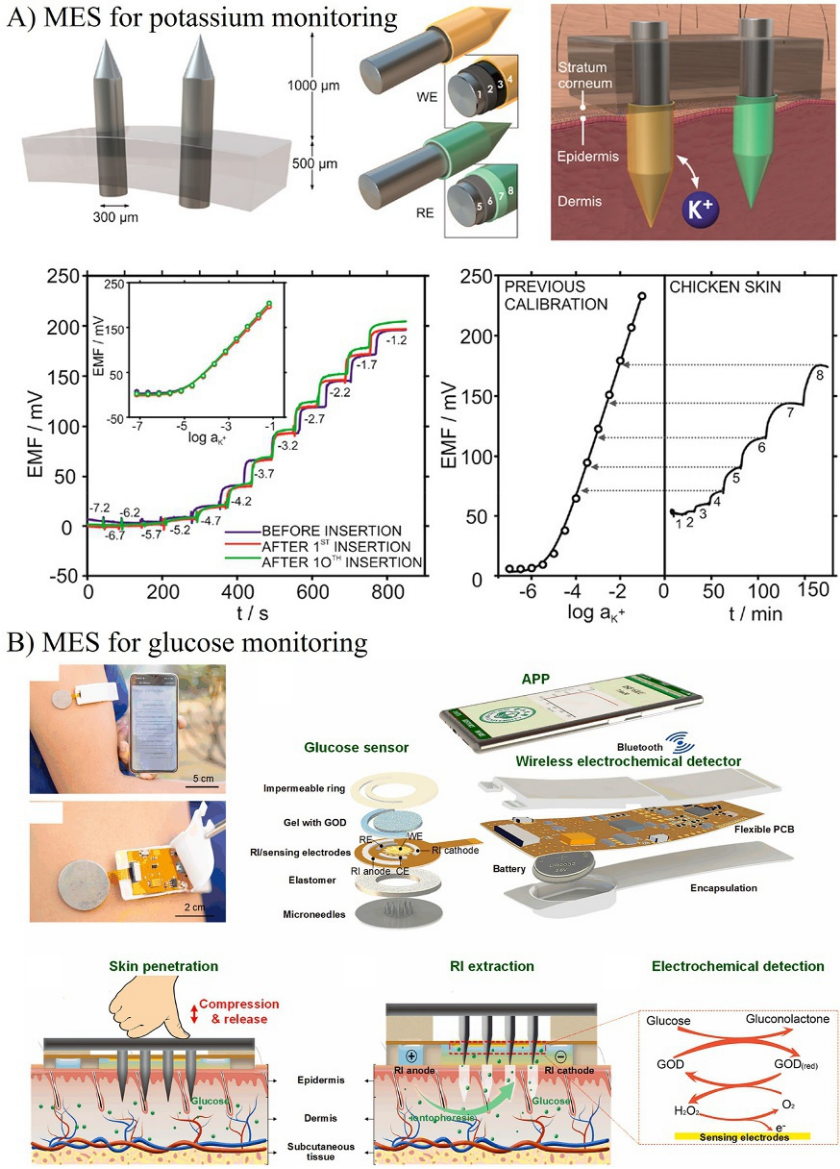

Fig. 6 MN-based electrochemical sensors for the monitoring of endogenous targets. (A) Potentiometric sensor for monitoring of potassium. (B) Amperometric sensor for glucose monitoring. *(Reproduced with permission from (A) M. Parrilla, M. Cuartero, S. Padrell, M. Rajabi, N. Roxhed, F. Niklaus, G.A. Crespo, Wearable all-solid-state potentiometric microneedle patch for intradermal potassium detection, Anal. Chem. 91 (2019) 1578–1586, https://doi.org/10.1021/acs.analchem.8b04877. (B) Y. Cheng, X. Gong, J. Yang, G. Zheng, Y. Zheng, Y. Li, Y. Xu, G. Nie, X. Xie, M. Chen, C. Yi, L. Jiang, A touch-actuated glucose sensor fully integrated with microneedle array and reverse iontophoresis for diabetes monitoring, Biosens. Bioelectron. 203 (2022) 114026, https://doi.org/10.1016/j.bios.2022.114026.)*

and alcohol, has been developed and validated in human tests [82]. In addition, nonenzymatic sensors have been constructed on solid MAPs [107,108]. Polymeric solid MAPs made of swellable materials such as silk have shown promise to monitor glucose. This approach consists of integrating functionalized wire-based electrodes on the MNs and performing the electrochemical analysis when the MNs are hydrated [80]. Hollow MAPs have also been used for ISF extraction and in situ analysis of glucose [63]. Another strategy is to embed a miniaturized electrochemical cell in a hollow MN in a way that the MN protects the sensor [109]. Following this approach, a wearable patch has been benchmarked with commercial Freestyle Libre [85]. Lastly, systems that employ solid MNs to pierce the skin and are subsequently retracted to allow the ISF to flow toward miniaturized electrochemical sensors have proven to be effective for glucose monitoring [110,111] (Fig. 6B). However, these systems might allow engineering complexity and hinder a rapid diffusion of the analytes toward the surface of the electrodes.

Lactate monitoring has attracted huge interest because it is a key molecule in sports medicine and clinical care. Lactate is a metabolite produced during the anaerobic metabolism of glucose in muscles. In addition, lactate can also be an indicator of several pathological conditions (e.g., cardiac diseases, infections, and cancer among other disorders). The common sensing strategy in electrochemical sensors for lactate detection is the enzymatic biosensor. Solid MNs have been employed to develop MES [86], as well as hollow MNs to extract ISF onto a planar sensor [112]. A recent report describes the use of MES in a phase I clinical study for the minimally invasive monitoring of lactate exhibiting potential toward the applicability of MN-based devices in real scenarios [113].

Diabetic ketoacidosis is a severe complication of diabetes mellitus with potentially fatal consequences. Therefore, monitoring ketone bodies along with glucose can be very valuable for diabetic patients. A MES able to monitor β-hydroxybutyrate via an enzymatic sensor using β-hydroxybutyrate dehydrogenase showed potential for continuous analysis of ketone bodies. The MES used hollow MNs filled with conductive pastes and functionalized with the respective enzyme to develop a multianalyte detection platform [114].

Cholesterol is a well-known target because high levels of cholesterol can increase the risk of heart disease. Thus, regular monitoring of cholesterol levels can provide information on a healthy lifestyle and better control of human health. For this reason, a MES for cholesterol monitoring was

developed based on solid MNs sputtered with gold, electrodeposited PANI, and platinum nanoparticles as the mediator along with cholesterol oxidase [115].

3.1.3 Proteins

Endogenous targets can also be produced by the irregular metabolism of certain cells. Cancer cells excrete biomarkers that can allow the early diagnosis or prognosis of specific cancers. Thus, MES for the detection or monitoring of cancer-related biomarkers is highly valuable for healthcare. In this direction, a MES for the detection of tyrosinase was developed [116]. This enzyme is highly produced by melanoma cells. The sensing strategy is based on the detection of tyrosinase activity by having immobilized catechol at the surface of MN-based electrodes. This work employed hollow MNs filled with conductive pastes for the modification of the MAPs. When the tyrosinase is present in the sample, the catechol is converted to benzoquinone, which is detected amperometrically with a current signal proportional to the level of the enzyme cancer biomarker.

The detection of breast cancer biomarkers is a relevant topic for early diagnosis of this disease due to its high prevalence. In this way, a functionalized solid MAP with antibodies toward epidermal growth factor receptor 2 (ErbB2) was developed for the extraction of the biomarker [117]. After incubation in ISF, the biomarker immunoreacts with the antibody forming the complex. Subsequently, the MES is removed for an ex situ analysis. In this case, the electroanalytical measurements are performed out of the body as a redox reporter is introduced to monitor the decrease in the current signal upon increasing target binding.

Among all the MES, solid MNs are the most used MAPs along with the enzymatic biosensor approach. Several articles also reported multiplexing capabilities expanding to multianalyte monitoring [82,114,115].

3.2 Exogenous targets

3.2.1 Virus and bacteria-induced biomarkers

MN-based devices for the monitoring of infectious diseases have been scarcely developed. The complexity of the sensing systems and low LOD needed to detect infection events are still a real challenge. However, the first MES has been reported for the monitoring of Epstein-Barr virus [118]. Interestingly, the electrochemical sensing strategy combines the synergic effect of clustered regularly interspaced short palindromic repeats (CRISP)-activated graphene biointerfaces on the MNs surface for the

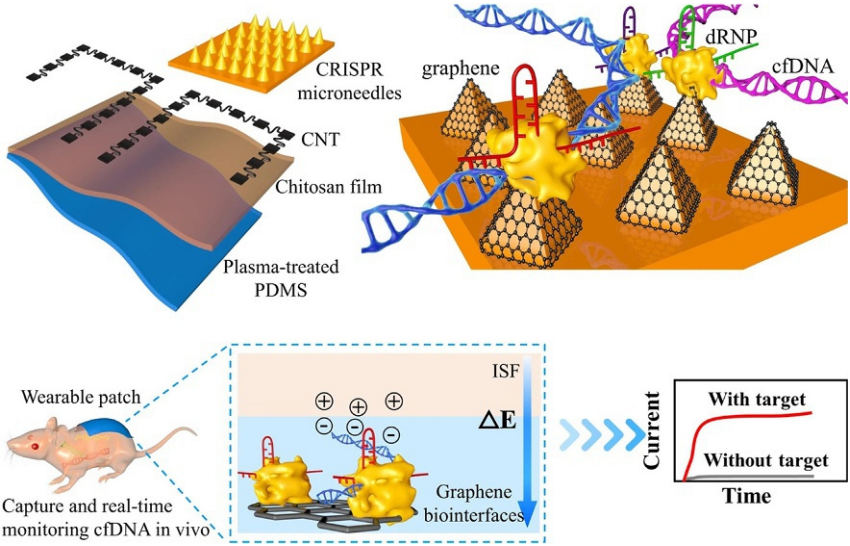

Fig. 7 MN-based electrochemical biosensor for the detection of cell-free DNA for diagnostic purposes. *(Reproduced with permission from B. Yang, J. Kong, X. Fang, Programmable CRISPR-Cas9 microneedle patch for long-term capture and real-time monitoring of universal cell-free DNA, Nat. Commun. 13 (2022) 3999, https://doi.org/10.1038/s41467-022-31740-3.)*

label-free in vivo monitoring of cell-free DNA in the femtomolar range (Fig. 7). Remarkably, the device exhibited a stable sensitivity for 10 days during in vivo tests. Moreover, the CRISPR-modified electrochemical technology was also tested for the detection of other DNA strands encoding for the monitoring of sepsis and kidney transplantation status.

3.2.2 Therapeutic drugs

TDM has proven to be an essential clinical practice to provide an effective treatment for specific disorders. The narrow therapeutic range of certain drugs makes TDM a necessity for optimal and personalized treatment in patients [119]. However, current methods rely on the extraction and analysis of the samples in a clinical setting hampering the daily routine of patients. Therefore, the decentralization of TDM by means of MN-based devices could dramatically enhance the lifestyle of the patient while providing optimal treatment [120].

Levodopa is a therapeutic drug widely used for treating Parkinson's disease. Importantly, this drug is an attractive target for electrochemical analysis as it offers several sensing approaches. For example, a hollow MAP filled

with conductive pastes was built for the direct oxidation of levodopa [121]. In addition, the use of tyrosinase enzyme allows its electrochemical detection by catalyzing its oxidation, which in turn can be directly reduced by applying a cathodic potential [121]. Similarly, solid MNs were functionalized with tyrosinase to develop an enzymatic MES along with a differential current response to avoid the interferent signals extracted from a nonfunctionalized MN electrode [122]. Another therapeutic drug for Parkinson's management was utilized as a model for MES (Fig. 8). A hollow MAP was developed for the direct oxidation of apomorphine [123]. SWV and chronoamperometry were employed to detect apomorphine in an in vitro setup. Besides, the direct electrooxidation of methotrexate by SWV has been recently reported using a crosslinked polymer to enhance the electrochemical signal while avoiding biofouling issues [120].

Antibiotics encompass a wide range of molecules. Their massive use can lead to resistance, which can become a dramatic issue for the effective treatment of the patient. Therefore, optimal use of antibiotics is needed to avoid the generation of antimicrobial resistance. For example, MES for the monitoring of beta-lactam type antibiotics has been developed by the immobilization of beta-lactamase enzyme on conductive solid MNs. The MES was able to monitor penicillin-G by monitoring pH changes at the surface of the electrode with a potentiometric sensor [124]. The successful arrangement

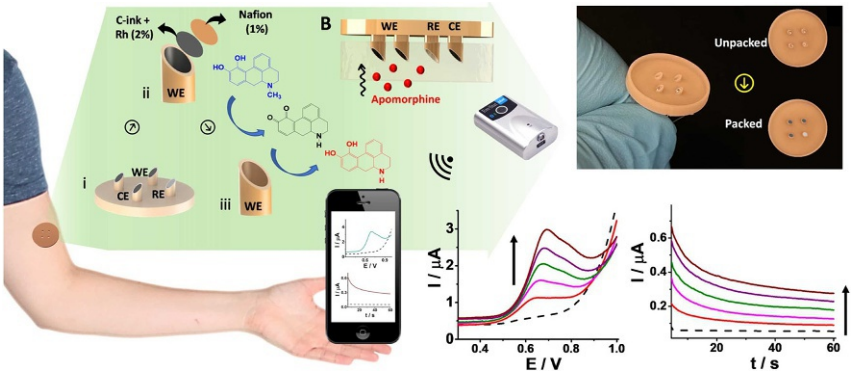

Fig. 8 MN-based electrochemical devices for exogenous targets: the case of therapeutic drug monitoring. Direct oxidation of an electroactive drug at the surface of the MN electrode [123]. *(Reproduced with permission from K.Y. Goud, K. Mahato, H. Teymourian, K. Longardner, I. Litvan, J. Wang, Wearable electrochemical microneedle sensing platform for real-time continuous interstitial fluid monitoring of apomorphine: toward Parkinson management, Sens. Actuators B Chem. 354 (2022) 131234, https://doi.org/10.1016/j.snb.2021.131234.)*

was tested in a human forearm exhibiting a potentiometric response upon administration of a penicillin dose [125]. Remarkably, the first clinical test in healthy volunteers was performed to validate the MN-based device by the administration of phenoxymethylpenicillin [126]. The proof of concept was validated with the extraction of extracellular fluid by microdialysis exhibiting similar concentrations of the antibiotic. Moreover, the continuous monitoring permitted the calculation of pharmacokinetic values proving the usefulness of the technology for real-time decision-making processes. Another MES was engineered to monitor two other antibiotics with narrow therapeutic windows (i.e., tobramycin and vancomycin). Solid MNs were also employed to build the sensing patch. For this MES, an aptamer-based sensing strategy was used taking advantage of the conformational change of the aptamer upon target binding. The conformational change alters the electron transfer rate of the redox reported linked to the aptamer (e.g., methylene blue) allowing for an in situ monitoring of the analyte. The authors performed a biocompatibility study of the materials, an evaluation of the penetration capability of the MNs, and a full ex vivo and in vivo test in mice. Interestingly, the authors studied the correlation of drug pharmacokinetics between ISF and blood during several dosages in the in vivo test.

The treatment administrated to cancer patients normally provides a highly harsh environment for the entire body leading to high toxicity not only for the cancer cells but also for the healthy cells. Hence, an optimal treatment according to personal pharmacokinetics could be truly relevant for patient side effects. Similarly to antibiotics monitoring, the aptamer-based sensing strategy was also used in solid MNs for the detection of antineoplastic drugs (i.e., irinotecan and doxorubicin) proving the versatility of the platform for multiplexing [120].

All in all, electrochemical sensing based on aptamers is a promising technology for MES allowing for the expansion of multiple analyte monitoring, which can be beneficial for the decentralization of TDM.

3.2.3 Illicit drugs

Illicit drug consumption is posing a tremendous issue in modern society. The high consumption rates of highly potent illicit drugs among the population are translated into increasing overdose issues. Therefore, a MES for the on-site detection of overdose could be valuable for first responders for proper antidote administration. A MES based on hollow MAPs was reported for the monitoring of fentanyl and morphine [127] as well as MDMA [128]. The modification of the conductive paste with nanomaterials

such as gold nanoparticles and reduced graphene oxide enabled the nanomolar detection of fentanyl showing promises for the real-time detection of fentanyl, which could prevent fatal effects in drug consumers [127].

3.2.4 Alcohol

Despite the health and societal issues that alcohol poses, alcohol consumption is widely spread among the worldwide population. One main problem is driving under the influence of alcohol, which can increase the probability to suffer a car accident. Thus, a monitoring system that can provide continuous digital information about the alcohol levels in the body can give information on when to drive or not the car. In this way, a hollow MAP was integrated with functionalized wires as electrodes for alcohol monitoring [84]. Another strategy employed solid MNs for alcohol monitoring in human subjects proving the reliability of MES for real-time digitalization of chemical information [82]. In both strategies, an enzymatic approach was employed using alcohol oxidase as the main driver of the electrochemical signal.

Among all the reported strategies for the monitoring of exogenous targets, the aptamer-based approach seems to be the most versatile technique to expand MES to other target analytes. However, new technologies based on CRISPR might open a new paradigm for the real-time sensing of nucleic acids. Details about the analytical parameters of several MES can be found in recent literature [20,21,64].

4 Remaining challenges and perspectives

In the last decade, a new army of MES has been described tackling the continuous monitoring of analytes that can be used for health management either by the monitoring of exogenous or endogenous species. However, there are still some challenges that MES developers need to address to expand MN-based electrochemical sensing to other analytes, and thus provide multiple sensors to be utilized in many use cases.

4.1 Monitoring of proteins and other macromolecules

MES have principally been designed for the direct detection of ions and small molecules by following well-established electrochemical strategies, such as enzymatic sensors and ion-selective electrodes, thus limiting the diagnostic and therapeutic applications of MES. Proteins are the building blocks of the body, as well as encode many biomarkers for multiple diseases.

Therefore, new electrochemical strategies for the monitoring of proteins will disrupt the decentralization of biochemical analysis for future health digitalization. These strategies need to overcome the need for sample treatment, meaning that the sensor delivers the analytical signal while wearing it. Aptamer-based sensors and MIPs labeled with redox reporters that change their conformation upon binding can satisfy the protein monitoring demand. Nevertheless, these approaches should also be reversible aiming for monitoring the analyte fluctuations.

4.2 In vivo testing, correlation ISF/blood, and validation

First, a proper analytical characterization of the MES needs to be executed in vitro, for example, the assessment of protein content in the matrix to assess the biofouling effect. Thereafter, ex vivo tests in porcine skin are essential to emulate real scenarios and minimize animal studies. Only when the device is properly characterized and fully functional in an ex vivo setup (e.g., calibration curve exhibiting the same parameters outside and inside the skin) to avoid a postinsertion calibration, animal testing should proceed. Finally, the validation of the MES in in vivo setups is essential to verify the reliability of the device. Here, the challenge is the proper extraction of ISF for the analysis with standard methods. The lack of standardized protocols for the validation of MES is also a demand that analytical chemists need to tackle. During the validation in animals or human subjects, the ratio of analyte free in ISF and analyte-bounded to protein needs to be considered for proper comparisons. The lack of knowledge on the free concentration of analytes in ISF hinders the proper validation of the technology, and it is indeed a research topic to investigate in near future.

4.3 Integration with other technologies

A fully functional MN-based device includes the MES, the electronics consisting of the electrochemical reader and the wireless transmitter, and a battery. Therefore, an interdisciplinary approach is needed to devise a fully integrated MN-based device. These miniaturized devices usually suffer from battery constraints. Therefore, the integration with self-powered technologies, such as energy harvesting and wearable energy storage systems, will bring the next generation of wearable self-powered analytical devices [129]. Biofuel cells are attractive as they can provide energy scavenging from molecules (fuel) in the ISF while showing analytical behavior. Triboelectric nanogenerators or thermoelectric harvesting can be also effective to power

wearable devices for analytical purposes [129]. In addition, the inclusion of electrochemical supercapacitors will assist in the miniaturization of the whole system.

MNs have been traditionally used for drug delivery [45]. Therefore, closed-loop systems for sensing and delivery based on MN technology [120] will empower self-sufficient treatments without the need for human intervention [119]. Closed-loop systems have been mainly reported for glucose/insulin management [103,111,130,131], and thus, future systems in other applications such as TDM [120] will furnish the patient with a radical improvement in the lifestyle. Finally, the use of AI with closed-loop systems will provide personalized treatment for optimal recovery of the patient while minimizing drug consumption.

5 Conclusions

A massive step forward in the development of MN-based electrochemical sensing technology has been exerted in the last decade. Innovative designs and configurations have shown the reliability of MES for the monitoring of biomarkers in human subjects. While the majority of MES monitors metabolites and electrolytes, a new trend is to engineer devices for TDM in ISF. Interestingly, future designs are advancing toward the detection of nucleic acids (e.g., DNA and RNA), which can unravel multiple diseases.

Essential aspects to consider when designing new strategies are (i) the presence of the biomarker in the ISF; (ii) the level of the biomarker in ISF, which can be lower than in blood depending on the size of the target; and importantly, (iii) its clinical relevance. However, this information is limited and usually not available as dermal ISF is an emerging research field whose mechanism of diffusion and partitioning of analytes needs still to be deeply explored.

The evolution of MN-based electrochemical sensing technology has been embodied in this chapter allowing the readers to have an outlook on the current state of the art of MESs. First, the importance of minimally invasive analysis of dermal ISF for health monitoring is entangled. Subsequently, the fabrication of MAPs and construction of MES have been detailed. The electrochemical techniques and the functionalization of MNs have been depicted to show the reader how to engineer a fully functional MES. Importantly, an overview of the proper electroanalytical characterization of the MES is exemplified. Thereafter, a variety of MES for the monitoring of different analytes with relevance in health monitoring has

been shown. Finally, current challenges and next steps for future MN-based devices have been described including the integration of emerging technologies such as energy harvesting systems. Overall, this chapter intends to be a guideline for researchers and engineers that want to develop the future MN-based electrochemical sensing technology for diagnostics and therapeutic purposes toward the next generation of health monitoring devices.

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CHAPTER 20

Future perspective, clinical translation, commercial challenges, and industrial success with microneedle-based products

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1 Introduction

The use of oral and hypodermic delivery methods is the most frequently used medication delivery modalities. However, there are a number of drawbacks while using these techniques, including presystemic breakdown, high pH levels and stomach irritants, discomfort from hypodermic injections, and a degree of unpredictability in drug absorption [1]. Despite the limited number of pharmaceuticals that may be delivered through the epidermis because of the stratum corneum's excellent barrier properties, the marketplace for transdermal pharmaceuticals is worth about $32 billion yearly. The primary drivers of growth include lower development costs, more market penetration resulting from improved patient acceptance, and the emergence of cutting-edge technology [2].

Microneedles (MNs), a transdermal method of administration that fuses the science and technology of patches for the skin with hypodermic needles, have seen a rise in research and development over the past several years. Since the needles are so small in the range of hundreds of microns they cause

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little or no discomfort. This method of administration goes beyond the drawbacks listed previously; is less costly, noninvasive, self-administered; and may also be utilized for prolonged drug delivery [3].

Clinical research and medication delivery experiments using the MN technology have shown favorable outcomes. However, if it causes patients' pain and discomfort, its popularity and benefits would be restricted. The care of both pediatric and adult patients may be substantially hampered by needle phobia, a dread of syringes, needles, injections, and intravenous accessories [4].

Needle phobia can range from minor to extremely incapacitating or even life-threatening. MN devices can be used in these circumstances due to their minimally intrusive and painless nature. According to a study, youngsters generally approve of this technique because there is no need for needles and there is less discomfort related to MN application [5].

However, it is important to address the MN system's drawbacks as early in the product development process as possible [6]. Significant advancements in microneedle based studies have been emphasized to assess the field's future direction, along with limitations that may prevent the technology from being fully utilized. In the following sections, we will be seeing the potential microneedles hold.

2 The potential prospects of microneedles in industries

As a result of the stratum corneum's barrier, different techniques to facilitate material transmission have been investigated. To lessen the permeation barrier of the skin, methods, including electroporation, ultrasonic enhancement, or chemical enhancers, have been used. However, there is a considerable benefit to using microneedles to distribute drugs since they may be utilized to create skin microconduits [7]. Also, there are five major types of microneedles and the following table shows their advantages and drawbacks (Table 1).

2.1 Electroporation

Electroporation is the deployment of a brief electric pulse to create temporary aqueous various tumor routes in lipid bilayer membranes [8]. Electroporation takes place when the transmembrane voltage exceeds a couple of hundred millivolts during electric field pulses ranging in duration from 10 Ms. to 100 Ms. Electroporation has been seen in metabolically inert systems, such as synthetic lipid membranes [9] and red blood cell ghosts [10], as well as in live cells and tissues [11].

Table 1 Various types of microneedles and their advantages and disadvantages.

Type of microneedle	Advantages	Drawbacks
Solid MNs	Sufficient amount of drug load and rigid structure	Skin injury is unavoidable, along with poor dose accuracy and poor biocompatibility
Coated MNs	Can be used for drugs with lower levels of dose	Low drug load and not adequately biocompatible
Dissolving MNs	Has controlled drug release and easier and simpler to manufacture	Dose limitation and only biodegradable materials can be used. Also lacks mechanical strength
Hydrogel MNs	Leaves no residue on the skin along with controlled drug release and provides easier production methods	Lacks mechanical strength
Hollow MNs	Provides improved delivery time along with higher drug load and precise dose advantage	Some auxiliary equipment is required. The tip tends to get clogged and is expensive. Has biocompatibility issues

2.2 3D printing

The adaptability, simplicity, high repeatability, and precision in the microscale that 3D printing is known for have sparked intense investigation into its application in the manufacturing of transdermal microneedle solutions [12]. 3D printing, which encompasses various technologies, enables the processing of many types of materials (e.g., polymeric, metallic, and ceramic) by inducing the right physicochemical processes. The similarity of 3D-printing methods is that they all involve the sequential creation of bonded layers in just one individual machinery, the 3D printer. Several methods have demonstrated promising results, either as a direct microneedle production process (direct printing) or as a tool for micromold creation (indirect printing) [13].

3 Applications of various types of microneedles-based products

To present, the majority of microneedle applications investigated have focused on medication and vaccine administration to the skin. The barrier

qualities of the topmost epidermal layer, the stratum corneum, hinder conventional transdermal administration [14]. To enhance skin permeability, many chemical, biological, and physical approaches have been investigated. Conversely, chemical and biochemical approaches do not appear to be universally useful for delivering biotherapeutics and vaccinations over the skin [14,15].

3.1 Biomedical applications

Due to first-pass metabolism, most biotherapeutic medicines that include proteins, peptides, hormones, and natural agents are difficult to give. As a result, despite the pain associated with needle insertion, hypodermic injection must be considered. Microneedle patches, an emerging product, are frequently recognized as potential options for hypodermic injection because they are not painful, safe, and simple for individuals to self-administer. Transdermal medication administration for disease therapy remains a popular use for microneedles [16].

Traditional cancer treatments, such as surgery, chemotherapy, and radiation, can cause severe toxicity and side effects, as well as tumor recurrence. A minimally invasive cancer treatment with microneedle patches is usually appealing due to its favorable controllability, ease of application, and prominent synergistic impact [17].

In the case of diabetes, the patient's compliance is likely to decline due to the complex and numerous daily injections. Additionally, regular mealtime administration dramatically reduces the risk of hypoglycemia [18]. Other illnesses were treated using microneedle patches that are used for transdermal medication administration. Alzheimer's disease (AD), which causes memory loss and language difficulties, is thought to be a chronic neuro-degenerative condition [19]. According to reports, compared to conventional therapies, analgesic microneedle patches are an efficient and convenient option for treating localized neuropathic pain [20]. Products made from microneedles may see quick growth and have wider possibilities in the next decades due to their simplicity and affordability.

3.2 Microneedles for drug and vaccine delivery

The use of microneedles in vaccination is particularly intriguing since it not only provides the anticipated benefits of simplifying vaccine administration and enhancing compliance among patients [21,22] but also permits vaccine targeting to the skin. It is well recognized that administering vaccines

through the skin has immunologic benefits over doing so by intramuscular injection, but there have not yet been any straightforward, dependable techniques for doing so.

This constraint is overcome by microneedles, which may be used to inject intradermally using hollow microneedles, as well as solid microneedle patches. The use of microneedles for vaccine administration has received the greatest research attention as a result of these potentials [23]. The great majority of microneedle-based studies involving human participants have taken into account how the technology is perceived, as well as its safety and usefulness [24]. With encouraging outcomes, the research has advanced to the administration of medications to people. The first medication to enter clinical research was naltrexone, which established the standard for further human testing [25,26].

The potential for improved vaccine component stability, particularly during production and storage, is a fundamental component of innovative vaccine administration techniques. The key barrier to vaccine stability continues to be encapsulation into evaporating MN or, alternatively, coating onto solid MN. The drying phases of manufacturing, linked to MN loading and formulation, are likely to result in loss of antigenicity, much like with other biological medicines [27]. An appropriate excipient must frequently be added when using solid MN methods to stabilize the antigen throughout the coating process and hence minimize activity loss [28].

Researchers in this field now place a high priority on MN product fabrication and oversight due to the trend toward improving the transdermal administration of bigger peptide, protein, and vaccine components, as well as conventional molecules. Schematic representation of manufacturing, transportation, and immunization of microneedles is shown in Fig. 1. Clinical trials are still a topic of discussion at many research institutions across the world [26].

3.3 Diagnostic applications

The main elements in illness diagnosis are clinically relevant analytes, including glucose concentrations, biomarkers, and ion concentrations. The enormous demand is progressively pushing toward less-invasive surveillance of these parameters in peripheral bloodstreams and interstitial fluid (ISF) using MNs. With blood and its contents altering in line with disorders, ISF includes many similar components [29]. The levels of the analytes in ISF can serve as an indicator of health status through the usage of indicators.

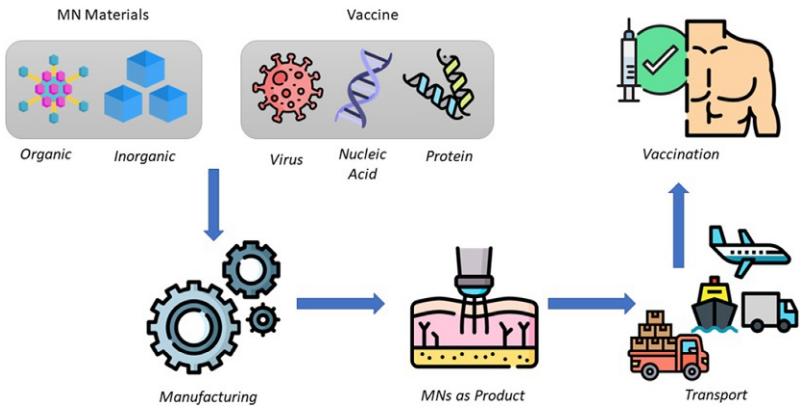

Fig. 1 Schematic representation of manufacturing, transportation, and immunization of microneedles.

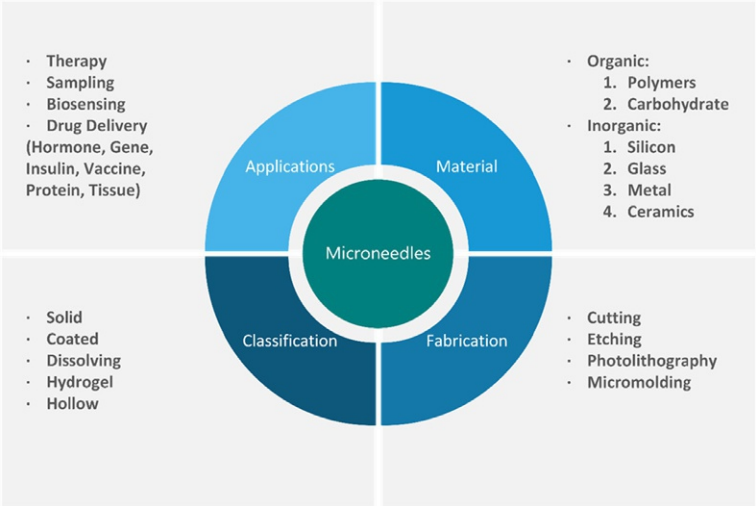

Fig. 2 Various aspects of microneedles.

MNs are frequently used with some analytical methods for the quantitative identification of clinically relevant factors [30]. Different applications of microneedles are shown in Fig. 2.

4 Clinical translations and advances

The medication delivery industry has undergone a revolution thanks to recent advances at the nano- and microscale. Because of reliability,

adherence by patients, and clever design, several kinds of microneedles (MNs) are being used for drug delivery applications. Despite increasing scientific and medical progress, attention should be directed toward improving the performance and durability of MNs. For MNs to be successfully translated into the clinic as an effective drug delivery method, the potential immune reaction or resistance should also be avoided.

4.1 Importance and methods of clinical translation

It is impressive how MNs of many sorts and materials have been successfully used in therapeutic situations, such as the administration of extremely effective and focused drugs or vaccines. MNs have so far found widespread use in illness diagnosis, painless disease monitoring, and sample extraction for biochemical analysis [31]. As an example, MN patching is employed to minimally invasively monitor illnesses by drawing interstitial fluid through the skin epidermis. The patients' experience during this procedure may be made pleasant and convenient with the help of MNs [29].

For diabetic patients receiving insulin injections (for type 1 diabetes or insulin-dependent diabetes), blood glucose measurement is a standard procedure. This procedure may be made painless and very practical for patients with the use of microneedles [32].

4.2 Advances and challenges faced in translation

It is noteworthy that before being used in clinical settings, microneedle items must be authorized in compliance with applicable standards [33]. Drug-free microneedles should be monitored and managed as medical devices, whereas drug-loaded microneedles should be managed as pharmaceutical preparations, according to the Food and Drug Administration (FDA), which defines microneedle equipment for use in cosmetics as Class II medical devices [34,35].

Guidelines for standardized microneedle design and use will be provided by a sound microneedle product development plan. The manufacturing process, materials, and designs of microneedles produced using 3D printing are distinctive from those produced using other techniques. Therefore, a separate analysis of the 3D-printed microneedle product is necessary [36].

A few microneedles have been applied in clinical settings despite the development of novel microneedle preparations and therapeutic plans. Advanced additive manufacturing technology, especially the SOPL technology, which enables quick modification of superior microneedles with good

structure, can not only realize customization but also offer a rapid, functional, reliable, low-cost, and adaptable production system, offering assistance for the prevalence of the translation of microneedles to the clinic [36,37].

5 Challenges faced in the commercial market

Before these MNs are widely employed in medical applications, a number of issues must be resolved. Some of the most important and fundamental problems, such as the transition from lab-based toward user-based MNs and their progress toward product commercialization, must be addressed on a considerable scale.

5.1 Current scenario and development

MNs are used in the current situation to enhance the penetration of micro- and macromolecules through the skin. Additionally, it comprises using the MNs patch to track the pharmacokinetics of drugs and immunologic biomarkers in dermal interstitial fluid [29] [38]. A biodegradable, self-applying MN patch with long-lasting action was created for contraception. Levonorgestrel hormone is constantly released via the Minnesota patch for improved contraceptive effectiveness [39].

In addition, MN, a keratin-derived substance, was created for therapeutic hair regeneration. Continuous hair follicle stem-cell activators are released by this MN patch [40]. However, theragnostic uses of MN for site-specific medication administration have recently been discovered and shown to be more efficient for illness diagnostics and prognosis [41]. For future use in animals and people, many MN fabrication and applications received clearance from regulatory authorities and ethical committees [42]. The creation of MNs in research labs and pharmaceutical businesses has been made possible in recent decades by the introduction of microfabrication manufacturing technologies.

5.2 Industrial and medical challenges

The volume of medicine to be put on and in the MN, the transport of hydrophilic macromolecules, contamination at the microscopic level, and a lack of mechanical toughness in biomaterials are all issues that need to be addressed while research and development of MNs [43]. Assessing the feasibility from the variety of MNs that are accessible targeting various illnesses looks tough and exacting as a result of the lack of accurate

mathematical representations of the drug release. Polymeric MNs have been the subject of mathematical modeling and simulation studies by academics in an effort to improve medication delivery [44].

Contrary to normal surface or systemic approaches, intravitreal injections offer the benefit of directly delivering medications to the desired spot [45]. However, this method of therapeutic delivery has a number of drawbacks that prevent it from being the best option, such as frequently received injections; essential tissue trauma; short half-lives of administered drugs; patient pain and discomfort; the need for special training; severe injection-related infections, such as endophthalmitis, hemorrhage, and cataract; structural harm to the organs; high drug-induced toxic effects; and higher costs. As a result, it is not the best option [46].

6 Industrial development, success, and future prospects

The area of microneedles has great potential to produce cutting-edge new products that will enhance wellness, appearance, and overall quality of life. This is due to the growing society, educational, and industry demand for research, development, and translation of microneedles, as well as the developing pipeline of microneedle applications, technologies, and clinical trials [47]. The expansion of fundamental knowledge seems to be fueling the growth of the industrial sector. MNs have a number of benefits over traditional needle-and-syringe delivery techniques for biological agents, particularly in terms of less discomfort, decreased chance of infection, and improved administration control [48].

A number of challenges need to be overcome before vaccination using microneedles succeeds in clinical trials and is fully commercialized, despite the fact that studies on microneedles being a transporter for vaccines are expanding every year [49]. The advancement of this technology into the first phases of clinical testing was made possible by the effective use of microneedles as vaccine delivery vehicles. In one study, 180 healthy people received the conventional influenza vaccination by microneedles, and the results revealed that patients who received the vaccine intradermally experienced local reactions like redness and swelling more frequently [50]. These side effects, however, were minimal, and a little dose of the vaccine administered by microneedles elicited the same degree of immune responses as an intramuscular injection of the entire dose.

Additionally, while transdermal delivery using microneedles now focuses mostly on the administration of pharmaceuticals, in future

generations, microneedles may be used to distribute cells locally to accomplish cell-based treatment. We predict that stem-cell administration using microneedles for organ regeneration and repair will become commonplace in the future [51]. Additionally, probiotics can be delivered by microneedles to the digestive tract to control the intestinal microbiome and cure various metabolic diseases. Before achieving this aim, certain pressing problems must be resolved, including how to ensure uniform cell dispersion within the microneedle, as well as how to sustain the survival of cells in the microneedle [52].

Despite the incredible advancements achieved in the field of microneedles, the creation of the next generation's multifunctional smart biomaterials based on microneedles is still urgently required for biomedical applications in the future. Microneedles are anticipated to ultimately progress toward large-scale production and practicality with the advancement of technology and science, making people's lives more convenient [53].

7 Summary and outlook

It is extremely likely that in the near future, commercially viable microneedle-based medication delivery technologies will be developed to broaden the transdermal marketplace for molecules that are hydrophilic and macromolecules. In MNs, a great deal of research is being done to create efficient therapeutic delivery systems using conventional drugs for novel therapeutic purposes. The quick realization of new knowledge seems to be fueling industrial advancement, making the outlook of the microneedle sector rather promising. Several clinical trials have shown that MNs are effective; however, there have been many more preclinical research. Under the condition that the issues with these devices are swiftly and logically resolved, experts from academia, business, and regulatory agencies are working together to assist MNs in moving toward safe and successful clinical deployment. It is anticipated that over time, microneedle-based technologies will enhance the detection, treatment, and control and raise the quality of life for people throughout the world in terms of their health. However, due to the MNs' complex and expensive manufacturing process, as well as a number of application-related issues, their clinical application may be delayed. In addition, cutting-edge manufacturing techniques, including micromachining and 3D printing, are anticipated to reduce prices and streamline production processes in the near future, leading, in due course, to patient and economic benefits.

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Design and Applications of Microneedles in Drug Delivery and Therapeutics

Edited by **Prashant Kesharwani**

Design and Applications of Microneedles in Drug Delivery and Therapeutics details the design, characterization, and applications of this novel drug delivery system. Microneedles offer several advantages over traditional oral and parenteral approaches due to their ability to be fabricated and altered in shape, size, and material type, and their convenience to be matched to specific treatment needs. This book covers the materials selection, design and fabrication approaches, and clinical translation of microneedles for a range of therapeutic applications, including: cancer therapy, gene and vaccine delivery, wound healing, and more.

This book is a forward-thinking resource for those working in the development of materials and devices for novel drug delivery.

Key Features

- Introduces the reader to skin anatomy and materials development with regard to microneedles as smart carriers for drug delivery, offering an interdisciplinary approach that will appeal to a wide audience
- Covers a broad range of therapeutic applications, from ocular diseases and photodynamic therapy to sensing and cancer treatment
- Provides an overview of the materials, design, fabrication, clinical translation, commercial aspects of microneedle development, and their applications in drug delivery

About the Editor

Dr. Prashant Kesharwani is presently working as an assistant professor at the Department of Pharmaceutics, Jamia Hamdard, New Delhi, India. He is also a recipient of several prestigious international and national awards, such as "USERN Laureate 2023" and "SERB-Ramanujan fellowship." Dr. Kesharwani has academic, industrial, and research experience at the international level (including the United States, Malaysia, and India). Dr. Kesharwani has more than 300 international publications in well reputed journals and 25 published international books. His h-index is 76 and i-10 index is 310 (on June 2024). He is a recipient of several research funding from various funding bodies. He has presented many invited talks and oral presentations at prestigious scientific peer-conferences, received international acclaims and awards for research contribution, supervised students/junior researchers and actively participated in outreach and scientific dissemination for the service of wider community.

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CHAPTER 11

Diagnosis and Potential Strategies to Discover New Drugs for the Treatment of Alzheimer's Disease (AD)

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Abstract: Alzheimer disease (AD) is most common cause of dementia, which is characterized by impaired cognitive and behavioural charateristics. Deposition of Aβ plaques and neurofibrillary tangs (NFTs) are the hallmark of AD. Generally it is a chronic disease where neurodegeneration, and loss of neuronal function arise earlier before it is diagnosed. Early detection of AD is important as it reduces the severity of the disease. In this regard, an effective tools/methods are available including CSF biomarkers, Magnetic Resonance imaging (MRI), Positron emission tomography (PET) but all these methods are painful and often cannot be afforded by the patients.

Therapy of AD includes inhibitors of choline esterases, and antagonists at NMDA receptors. From the studies it is shown that these drugs just offer relief from symptoms rather than alleviating the progression of disease. Multiple pathological processes contribute for AD, like oxidative stress, dysregulation of neurotransmitters, inflammation of neurons, aggregation β-amyloid, phosphorylation of tau protein. It is essential to target multiple causes for an effective outcome in the treatment of AD. Early diagnosis is also crucial as it reduces disease progression thereby cost involved in AD therapy.

This review focuses on non-invasive, patient affordable diagnosis methods and also potential targets to discover new drugs beyond conventional and available drugs.

Keywords: Alzheimer's disease, Diagnosis, Non-invasive, β-amyloid and Tau protein.

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INTRODUCTION

Alois Alzheimer a German physician described first about Alzheimer's Disease (AD) in 1907 [1]. Dysfunction of Cognitive and non-cognitive features are the characteristic symptoms of AD. Cognitive impairment symptoms includes dementia, impediment of language and dysfunction of execution. Whereas psychiatric, disturbance in behaviour, depression, hallucnations, delusion, agitation are the non-cognitive impairment symptoms [2].

A study from The Global Burden of Diseases, Injuries and Risk Factors reported that around 40-50 million people across the world are living with AD and other forms of dementia. AD accounts for 2.4 million deaths globally and is the fifth major cause of death [3]. It is estimated that approximately 100 million cases of AD by 2050.

The main causes of AD are the loss of neurons and altered synapses due to the deposition of β-amyloid protein, destabilisation of microtubules by hyperphosphorylated tau protein, inefficient cholinergic neurotransmission, and the presence of other diseases like diabetes, hyperinsulnaemia, vascular diseases, neuronal inflammation, altered apolipoprotein gene.

Though it is a major public health problem currently five drugs are available to treat AD and are categorized into two classes. One is inhibitors of cholinesterase (Tacrine, Donepezil, Rivastigmine, Galantamine) and the other one is antagonist at N-methyl-D-aspartate (NMDA) receptor (Memantine). Although new drugs are developed against AD, including small molecules and immunotherapies, but they failed to exhibit different activities between drug and placebo, and moreover, exerted intolerable toxicities [5].

Nearly one hundred compounds have failed during clinical testing of the drug development phase [6]. Monoclonal antibodies are thought to be a promising agents against the amyloid cascade, Ex: Bapineuzumab [7], Solanezumab [8] but were plagued by side effects such as vasogenic edoema. The γ-secretase enzyme inhibitors [9] like Avagacestat [10] and Semagacestat [11] were found to increase the risk of skin rashes, diarrhoea, nausea, and skin cancer.

Symptomatic relivers (neurochemical enhancers) like Encenicline tends to boost cholinergic response at its receptor, while Idalopiridine tends to increase ACh release in the brain. However, these medications demonstrated substantial gastrointestinal toxicity, leading to their withdrawal from phase 3 clinical trials [12].

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The development of the primary neuropathological features of AD is influenced by some pathological processes that manifest years before the emergence of amyloid plaques and NFTs. Cellular senescence, oxidative stress, neuroinflammation, decreased neurogenesis, and altered proteostasis are some of these changes [13 - 15]. Many studies have shown how these early changes contribute to the acceleration of cognitive decline, a rise in Aβ load, and tau hyperphosphorylation and neuronal death. The beginning and escalation of the aforementioned degenerative alterations indicative of AD are thought to be mediated by a number of signaling pathways [16–18].

Therefore, we have focused on the PI3K/AKT and Wnt signalling pathways and how their roles are altered in many facets of AD as well as how they interact, particularly in causing apoptosis, angiogenesis, cell division, and viability in the metabolism of the CNS cells.

Rather the symptom remission, disease modifications are the main emphasis of researchers in AD. Since AD is a multifactorial disease it is important to target different aspects. But currently approved drugs fails to act on multiple steps involved in AD.

Diagnosis of AD is equally important to that of therapy because it reduces the risk of disease, cost burden on patients. So it is essential to understand diagnosis and differnet targets for effective outcome in the treatment of AD.

The existence of cognitive impairment, a key component of the dementia profile,may be identified through a combination of the patient's medical history, clinical examination, and an objective cognitive assessment, such as a thorough neuropsychological evaluation or a quick mental assessment [19].

Diagnosis criteria for Alzheimer’s disease are increasingly being updated to include biomarker tests for conditions such as amyloid-protein buildup, neuronal damage, synaptic dysfunction, and neuronal degeneration [20]. Also in identifying stages of AD, CSF biomarkers, and imaging tests are emerging as an essential tools [21].

Hence, the objective of this chapter is to present an overview of potential strategies to discover new drugs in the treatment of AD with respect to important hypotheses, signaling pathways also provide information on diagnosis methods in Alzhiemer’s disease.

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Pathogenesis of AD and Potential Strategies to Discover New Drugs

Mild AD frequently manifests as memory loss, personality changes, and impaired reasoning, poor judgement, and the inability to carry out routine daily duties, among other symptoms [22]. The main clinical and co-pathological signs of AD are the diminished activity of acetyltransferase, the development of amyloid plaques, also known as senile plaques, in extracellular locations, while hyperphosphorylated tauProtein aggregates to form neurofibrillary tangles (NFT) in infected neurons' intracellular sections [23]. The currently licenced medications only affect one target (one drug, one target; ODOT), while recent research has focused on several therapeutic approaches to create medications that can affect many targets [24 -26]. Despite extensive research, the exact cause of Alzheimer's disease remains unclear, but several hypotheses have been proposed to explain its pathogenesis. These hypotheses have been instrumental in guiding the development of a new drug for the treatment of Alzheimer's disease.

Cholinergic Hypothesis: In comparison with healthy brains of cerebral cortex there is reduction in choline acetyltransferase activity and level of acetylcholinesterase. Biochemical exploration of biopsy tissue and AD patients' brain tissue at post-mortem showed diminished activity of acetylcholine transferase, synthesis of acetylcholine, reuptake of acetylcholine and release of acetylcholine [27]. Decline in neurons of cholinergic system, cholinergic neurotransmission deprivation in the cortex of cerebrum and other regions of brain results in cognitive impairment [28].

Scientifically, it was investigated by Davies P. and Maloney AJ. that cortical, Amygdala and hippocampus choline acetyl transferase activity in Alzheimer's disease brains were significantly diminished. Acetylcholine esterase activity is significantly lower than normal brain activity in all other sections of the cerebral cortex, and in the same regions of the cerebral cortex where cholineacetyl transferase activity is also lowered. The regions of the cerebral cortex that exhibit the greatest decreases in cholineacetyl transferase and acetylcholine esterase activity are those that have the highest density of neurofibrillary tangles.

It is further noted that there is no loss of aromatic aminoacid decarboxylase, tyrosine hydroxylase, monoamine, and dopamine oxidase activities in AD and support the idea that selective destruction of the cortical cholinergic system is a key characteristic of this condition.

There is a scope to develop potential new drugs which can improve cholinergic neurotransmission *via* alteration of acetylcholinetransferase, acetyl cholineesterase and provides symptomatic relief in treatment of AD.

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Tau Hypothesis: An another hallmark of AD which is made up of tau protein (Tau proteins belongs to family of microtubule associated protein) is existence of intraneuronal neurofibrillary lesions [29]. Tau protein exists in six isoforms and also comprises a component which play an important role in microtubule polymerization and stabilization called microtubule binding domain (MBD) which is of highly conserved three or four repeats of eighteen amino acids [30]. Tau proteinphosphorylation is evolutionarily supervised from foetal to adult stage. In case of immature brains phosphorylation of Tau takes place at six to eight sites whereas, two to three phosphorylation in adult brain. By modulating serine/threonine phosphorylation, binding ability of tau protein can be significantly regulated [31]. The MBD of tau comprise repeats of four sequence (R1–R4) of serine (S) and threonine (T) followed by proline (P). Glycogen synthase kinase 3 (GSK-3β), cyclin-dependent kinase (cdk5) and its activator subunit p25 or mitogen-activated protein kinase (MAPK) causes hyperphosphorylation of all these amino acids [32]. In additionAkt, Fyn, protein kinase A (PKA), calcium–calmodulin protein kinase 2 (CaMKII) and microtubule affinity regulating kinases (MAPK) (nonproline directed kinases) are also involved in hyperphosphorylaton of tau protein. Hyperphosphorylated tau protein diminishes the affinity towards microtubule [33].

A cytosol of AD brain shown an abnormal augment with hyperphosphorylated tau protein.Neurofibrillary tangles (NFT) are formed as sequel to tau proteins phosphorylation into paired helical filaments and straight filaments. Pathological disturbance in structural and regulatory functions of the cytoskeleton can be seen as a consequence to loss of normal function of tau protein as depicted in Fig. (**1**). Which in turn leads to neurodegeneration, altered synaptic function, axonal transport, synaptic function and maintenance of appropriate morphology in neurons. Therefore, hyperphosphorylation, misfolding and aggregation of tau resulted from several pathogenic events [34]. Frontotemporal dementia and parkinsonism (FTDP) correspondent to chromosome 17 caused by mutation in tau gene. In patients with FTDP-17filamentous inclusions made by hyperphosphorylated tau could be seen [35]. Hyperphosphorylation of tau and regulation of tau kinases and phosphatases are closely associated with each other. Glycogen synthase kinase 3β (GSK-3β), cyclin dependent protein kinase 5 (cdk5), cAMP-dependent protein kinase (PKA) and stress-activated protein kinase can cause phosphorylation of taudemonstrated from *in-vitro* studies [36].

Hence siege of kinase enzymes responsible for hyperphosphorylation of tau would be an approach to block tau deposition. Another strategy to diminish NFT is dephosphorylation of tau carried out by upregulation of number of phosphatases (PP) 1, PP2A, PP2B and PP2C.

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Amyloid Cascade Hypothesis: Dysfunction of neurons and cell death in brains of AD patients occurs as a result of neuritic injury, formation of NFT *via* tau protein by the accumulation of amyloid beta (Aβ) plaques [37 - 39]. It is believed that Aβ plaques are principal source of AD. A widely distributed transmembrane amyloid precursor protein (APP) produces Aβ peptides (possesses 39–43 amino acid residues), key component of Aβ plaques bysequential enzymatic action of β-secretase and γ-secretase.

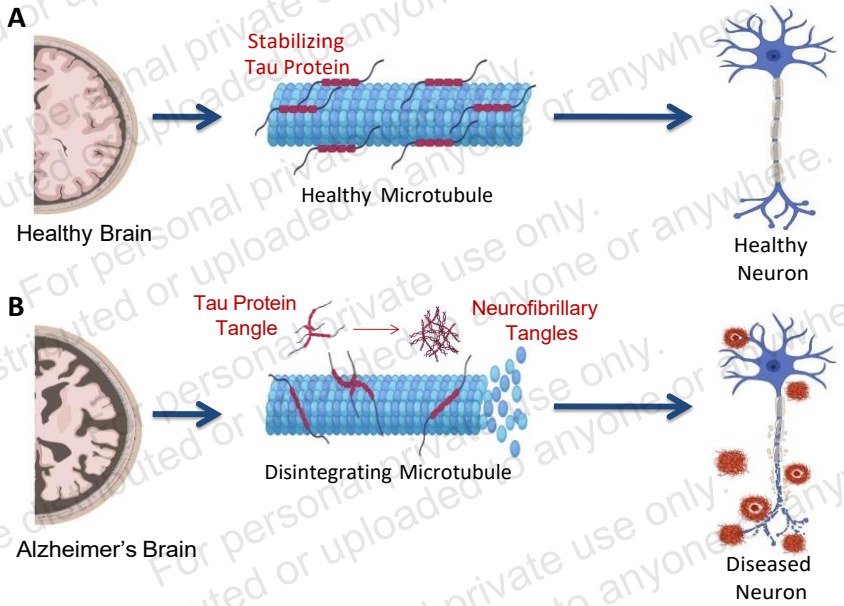

Fig. (1). Healthy and disintegrating microtubule in Alzheimer's disease brain.
This image was created using Bio-render tool, to differentiate healthy and brain with AD. From Athar T, Balushi KA, Khan SA. Recent advances on drug developmen and emerging therapeutic agents for Alzheimer's disease. Mol Bio Reports 2021; 48: 5629-45.

The amyloid precursor protein (APP), belongs to type I transmembrane glycoprotein comprises of 695–770 amino acids. A soluble extracellular fragment, sAPPα is formed by the cleavage of APP by an extracellular protease called as α-secretase. In addition a soluble extracellular fragment called sAPPβ and a cell-membrane-bound fragment (C99) is formed by the action of an aspartyl protease known as β-secretase 1 (BACE1) on APP. Further, C99 is cleaved by γ-secretase (complex of four proteins: presenilin, nicastrin, anterior pharynx defective 1 and presenilinenhancer 2). An intracellular peptide called amyloid intracellular domain formed by cleavage of γ-secretase. Initially Aβ aggregates to form oligomers, protofibrils, fibrils andultimately into plaques which is another hallmark of AD. Accumulation of amyloid-β (Aβ) peptide starts as earlier as clinical symptoms occurs [40 - 45].

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Hence potential drugs can be discovered which helps to alleviate Aβ monomers, oligomers, aggregates and plaques formation.

Signalling Pathways in AD and Associated Enzymes as Drug Targets for Treatment of AD

The brain with up to thousands of other neurons forming interrelation approximately 100 billion nerve cells (or neurons), which is known as synapses.

Cell signalling is the transmission of molecular signals from a cell's exterior to its interior. Signals received by cells must be transmitted effectively into the cell to ensure an appropriate response. This step is initiated by cell-surface receptors .In the management cognitive behaviour many signalling pathways play a major role some of them have illustrated below [46].

PI3K (phosphatidylinositol 3-kinase) /AKT (protein kinase B) Signal Pathway

A signal transduction pathway that contributes to existence and expansion in response to extracellular signals is known as Akt signaling pathway or PI3K-Akt signalling pathway, PI3K and Akt are involved in it. In CNS these proteins portay a dramatize role. Enzymes entranched on PI3K/AKT signal pathway serves as an effective target for the discovery of new drugs as microglia activation, dopaminergic, hippocampal, cortical neurons protected by them [47]. Hence upregulation of genes codes for proteins involved in PI3K/AKT signalling pathway is important.

There are three classes of PI3Ks categorized based on their structure and substrate selectivity.Class I isoforms are excited by cell surface receptors and are consisting distinct component of p85 and p110 catalytic component. Two proline-rich regions and a Src Homology 3 (SH3) domain are present at the amino terminus of P85 whereas non-coding region and two SH2 domains attaches to p110 makes up basal terminus. Class IA (PI3Kα, β and δ) and class IB (PI3Kγ) are the auxiliary segments of class I isoforms [47].

P0110α, β, δ are the catalytic subunits and p85α, β, c are the regulatory subunits of class-IA. Regulatory isoforms of p101 or p87 yoked with p110γ catalytic subunit forms class-IB. When a transmembrane glycoprotein receptor called tyrosine kinase bound by an extracellular ligand PI3Kα, β and δ get activated in the same way G protein coupled receptors (GPCRs) and Ras family GTP enzymes activation stimulates PI3Kγ [48].

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PI3KC2α, 2β, 2γ are the isoforms of class-II PI3Ks which necessitates binding with membrane and additional signals for its activation. Vacuolar protein sorting 34 (VPS34)is the only class-III isoform and is significant for early endosomes plasma membrane [49]. Hence we can target significant raise in the expression of PI3Ks a key element involved in the supervision of signalling pathway and elevates programmed cell death, transformation, angiogenesis, number of cells. Fig. (**2**) represents that role of PI3K/AKT isoforms in regulating damage of neuronal cells.

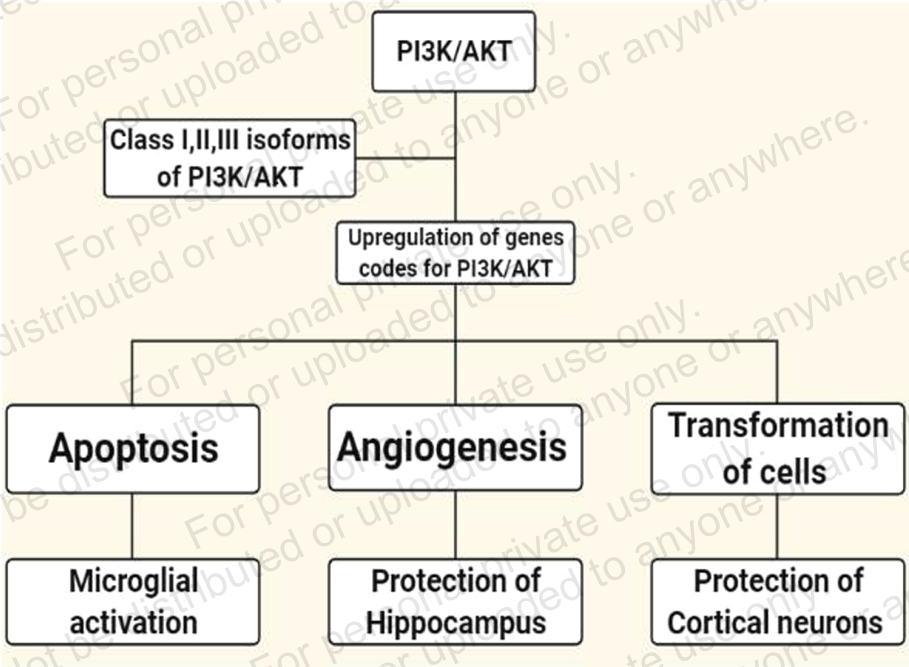

Fig. (2). Role of PI3/AKT pathway in AD.
This image was created using Bio-render tool, to understand importance of PI3K/AKT pathway to discover new molecules. From This image was created using Bio-render tool, to differentiate healthy and brain with AD. From Thorpe, L. M., Yuzugullu, H., and Zhao, J. J., PI3K in cancer: divergent roles of isoforms, modes of activation and therapeutic targeting ,Nat. Rev. Cancer.2015: 15 (1), 7–24.

Wnt Signalling Pathway

Mitochondria play a pivotal role in reducing apoptosis by conserving production of energy and calcium homeostasis. But there are many pathways that increases mitochondrial genes transcription as a consequence to inflammation and oxidation in the cell [50]. Some of those signalling pathways are activation of the Sirtuin1(silentmating-type in formation regulator 2homolog1)/peroxisome peroxisome proliferator-activated receptor gammaco-activator1- α (Sirt1/PGC-1α) axis, Mechanistic target of rapamycin (mTOR) complexes, Wnt signal

transduction pathways, 5-adenosine monophosphate-activated protein kinas(AMPK) [50].

Wnt Signalling pathway take part in programmed cell death, cell division, proliferation, adhesion, viability in metabolism of CNS cells.

Frizzled transmembrane receptors are present on surface of the cell where Wnt binds leading to induction three downstream signaling pathways.

- Canonical Wnt pathway – through β-catenin it regulates gene transcription.
- Non-canonical pathway- modulation of intracellular Ca2+ release.
- Wnt cell polarity- here the main role is of Jun N-terminal kinase (JNK) [51, 52].

Wnt Signaling Protects Synaptic Integrity from Aβ Toxicity: Most of the Wnt constituents are influenced in AD like β-catenin levels reduced which carries presenilin-1 (PS1)-inherited mutations [53]. Canonical Wntsignaling inhibition happens when cultured hippocampal neurons are exposed to Aβ [54, 55]. Aβ protein induces a Wnt antagonist, Dickkoff-1 (Dkk1)in hippocampal neurons [56], AD induced brain sample from transgenic animals and some of the patients with AD showed elevation in Dkk1 level whereas Dkk3 levelswhich is greatly related to Dkk1 significantly increased in plasma and CSF of AD patients [57 - 59]. An AD risk factor called apo-lipoprotein E (apoEε4) inhibited by Canonical Wntsignalingpathway [60]. Disease will be progressed due to variation in genes code for low-density lipoproteinreceptor-related protein 6 (LRP6) [61]. Active pre-synaptic sites and synaptic proteins reversible reduced by Dkk1. Cognitive impairment and tau-phosphorylation resulted from synaptic disassembly at pre- and postsynaptic sites [62, 63].

Therefore, induction of the non-canonical Wnt/PCP-JNK pathway driven by Dkk1 leads to Aβ amyloid toxicity responsible for late onset of AD [64]. As depicted in Fig. (**3**), Dickkof-1 which can activate non-canonical Wnt pathway causes amyloid toxicity which is responsible for late onset of AD.

Hence blockade of Dickkoff-1 (Dkk 1) an antagonist of Wnt helps in inflection of synaptic development and in the alleviation of AD [65].

Biomarkers in AD Diagnosis

Thet threat of a drug reinforcing is soaring, and biomarkers epitomize a heartening means of lowering the distress and recreate a critical role in Alzheimer's disease (AD) drug development. There is a trend towards incorporating biomarker tests into the diagnosis of AD criteria, such as amyloid-β

protein accumulation, neuronal injury, synaptic dysfunction, and neuronal degeneration [66 - 68].

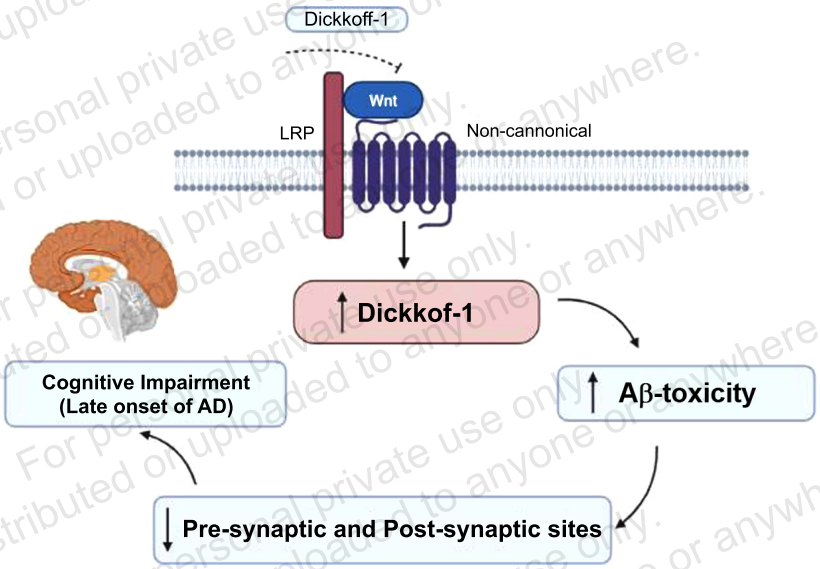

Fig. (3). Cognitive impairment by non-cannonical wnt pathway.

A biomarker is a persona that is objectively deliberate and quantified as a marker of conventional biological processes, pathologic processes, or biological comeback to a therapeutic medication [69]. Biomarkers constitute degrees of genes like genomics, transcriptomics, proteomics, metaboliomics, lipidomics, imaging, and blood electrocardiograms or gauging of organ function [70, 71]. Amyloid (A), tau(T) and neurodegeneration (N) research frameworks use biomarkers to diagnose AD [72].

Appeal with biomarker consequence are shorter and lessened than those acquired to show case clinical favour and pivotal to apprehend the biological collision of an agent and inform go/no-go decisions. Biomarkers comfort and portay the base-line state, a disease process, or a counter attack to treatment. BEST(Biomarkers,Endpoints, and other Tools) resource—to provide a shared vocabulary for biomarker discussions, approved by National Institutes of Health (NIH) enhanced a universal appendix of biomarker-related terminology [73].

Table **1** represents that some of the fluid biomarkers in AD patients and co-relates stage of disease/disease progression.

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Table 1. Exhibits an outline of the expressions convinced by biomarkers in AD drug development. Table that depicts role of various Bio-markers from Cummings J. The Role of Biomarkers in Alzheimer's Disease Drug Development. Adv Exp Med Biol. 2019;1118:29-61.

Role in Trial	Biology Identified	Fluid Biomarker	Brain Imaging
First to deduce participant and findings	Inhabitance of disease-type and observed pathological changes	Ratio of Low CSF Aβ42 or CSF Aβ42/t-tau	Confirmed imaging of amyloid
Target engagement	amyloid production found to be reduced	ReducedAβ42production	Confirmed imaging of amyloid shown reduced Aβ
At the phase of Support for disease alteration	As compared to placebo Reduction in portion of neuro degeneration	CSFt-tau found low	Drug-placebo difference infavor of active treatment for FDG PET hypo metabolism or MRI atrophy
Analytic accumulation	The status of ApoE-4carrier	ApoE genotype	-
Damaging effect	Liver and blood got effected	CBC and blood liver function	-
-	Monoclonal antibodies used in Production of ARIA	-	MRI Imaging used for monitoring

Role of CSF Biomarkers: In current clinical practices proteins such as Aβ42, total-tau (tau) and p-tau are the most sizable elements of amyloid plaques as well as tangles of neurofibrillary and ordinarily contemplate the gold standards for AD CSF biomarkers. It has been observed that CSF is informed a chief source for AD biomarkers because numerous proteins and its metabolites in CSF are directly going and reflecting the internal milieu of the brain. It has been comprehend for the collection of CSF, which makes it somewhat more invasive than a blood draw a lumbar puncture (LP) is requisite [74, 75]. Under the supervision of experienced clinicians, it is usually not painful.

Aβ42: A well-known 42 amino acid peptide that is Aβ42 formed from the fining of the precursor of amyloid protein. It has been noticed that multiple lengths of Aβ peptides are available and in that category the 42 amino acid form is the most bountiful section of amyloid plaques [76]. An individual who is significantly showing reduced levels of CSF Aβ42 in diagnosis with AD, as compared with logical normal, age-matched individuals, is one trademark of amyloid plaques [76]. From previous studies it is noticed that the low level of CSF Aβ42 is applicable as a marker that is going to be used for forcast the clinical disease

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progression and out lay of cognitive decline, particularly in the quick clinical stages of the disease [77 - 79]. Although, it came in notice that Aβ42 alone is not an adequate biomarker for the diagnosis and prognosis of AD and also observed nor does it mark the presence of other AD pathology [77, 78].

Blood Biomarkers: Under expression of biomarkers of AD in plasma and serum noticed also significant difference in total protein of plasma and serum in comparison with CSF which interferes with detection of analyte, so blood biomarkers are not promising tool to diagnose AD [80]. However, Aβ42 and tau in plasma can be considered as biomarker which are hallmarks of AD.

- **Plasma Amyloid-β42:** In plasma of some individuals with AD showed increased levels of Aβ42 or Aβ40, whereas no change in certain individuals. Although elevated level of plasma Aβ42, or a high Aβ42/Aβ40 ratio shows increased risk for future AD [81]. Therefore, early diagnosis by this biomarker could serve as efficient method.
- **Plasma Tau:** Dissension of plasma tau in patients with AD due to extremely low level compared with CSF [82], plasma tau is not an efficient blood biomarker in AD [83], but alternate assays and ultrasensitive methods are developing to get functionality of measuring the plasma tau [83].

Novel Blood Biomarkers: In spite of difference between plasma and CSF Aβ, tau there are few distinctive biomarkers present in blood like C-reactive protein and antibodies in serum which could recognize selective peptoid ligands in AD compared to non-AD samples. So Novel plasma biomarkers serves promising diagnostic tool in AD [84, 85].

MRI Biomarkers: It came in to focus that most commonly imaging biomarkers for AD is functional MRI (fMRI). When a person is engaging a task or while resting, deviation in magnetization between oxygen-rich and oxygen-poor blood can be deliberated by fMRI, which is useful to make out changes in association between areas of the brain. On task- based assessments fMRI differences can be seen between control subjects and individual with MCI according to some studies [86].

It has been observed presently, Food and Drug Administration has approved few diagnostic software programs for volumetric MRI and the process of analyzing scans is tedious and demanding. Hence so many studies are going on volumetric MRI. In whole brain or either in targeted areas Percentage of the size of a brain region allows for unearthing of atrophy [87 - 89].While reviewing papers, a marked decrease in volume is observed in AD; this is seen both in normalized whole brain volume and in specific areas such as the hippocampus and entorhinal cortex [89].

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Non-invasive and Cost-effective Methods to Diagnose AD

Neurophsychometric tests:-

There are three stages before the development of dementia as per Neurophsychological assessment:

1. Asymptomatic Pre-Mild Cognitive Impairment (MCI), 2. Symptomatic Pre-MCI and 3. MCI [90].

Episodic memory deterioration has been identified as a distinctive aspect of preclinical AD in cognitively normal people who later developed AD dementia. The transition from healthy ageing to preclinical AD and into the clinical phases of AD involves a decline in other cognitive areas as well, including visual-spatial,global cognitive performance and execution.

It is noticed that deterioration in language, executive function, and episodic memory in cognitively healthy adults with a high fibrillar amyloid burden.

Therefore, early diagnosis of AD, even in cognitively healthy individuals, provides an idea about the occurrence and progression of AD. In this view, Table **2** summarises neuropshychometric tests as early, non-invasive, and patient-friendly diagnosis methods.

Table 2. Neuropsychometric and clinical tests to diagnose AD.
Table represents assessment tests for different kinds of memory. From Chapman RM, Mapstone M, Porsteinsson AP,Gardner MN, McCrary JW, DeGrush E *et al*., Diagnosis of Alzheimer's disease using neuropsychological testing improved by multivariate analyses. J Clin Exp Neuropsychol. 2010 Oct;32(8):793-808.

Characteristic	Assessment Tests
Episodic Memory: able to recall past personal experiences at particular place and time	a) Logical Memory sub-test from the Wechsler Memory Scale [91] b) The California Verbal Learning Test, now in its second revision (CVLT-II) [92] c) The Free and Cued Selective Reminding Test (FCSRT) [93]
Assessment of subjective memory complaints	Subjective Cognitive Questionnaire/Simple questions [94]
Assessment of late-onset depression	Analyse Elevated depressive symptoms [95]
Speech testing	Automatic Speech Analysis and Recognition (ASR) test [96]
Olfactory testing	Tests to measure odour memory, Threshold, Identification and Discrimination [97]
Eye testing	Visual field, color vision, contrast sensitivity test [98]

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(Table 2) cont.....

Characteristic	Assessment Tests
Gait testing	Simultaneously performed tasks [99]

CONCLUSION

This review summarizes the potential targets to discover new drugs and also idea about non-invasive, patient friendly diagnostic methods for the detection of Alzheimer's disease. As AD is a multifactorial disease but currently available drugs fails to target multiple steps. There are only two categories of drugs to treat AD. Opportunities are there to discover new drugs which can act on various events of Pathogenesis, signalling pathways thereby prevent occurrence of disease. We also discussed understanding of key targets and development of potential therapeutic agents for the treatment of AD. Targeting the causes of disease minimizes worsening of condition also exerts cost cutting effect on patients.We also included an alternative diagnostic approaches which are non-invasive.

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ARTIFICIAL INTELLIGENCE IN PHARMACEUTICAL TECHNOLOGY

Volume 3

Editors
Dr. Akhil Sharma
Dr. Shaweta Sharma
Dr. Praveen Kumar

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ARTIFICIAL INTELLIGENCE IN PHARMACEUTICAL TECHNOLOGY

Volume - 3

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Chapter - 1
Virtual Healing: Unveiling the Power of E-Consultation in Modern Healthcare

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Chapter - 1

Virtual Healing: Unveiling the Power of E-Consultation in Modern Healthcare

Ashim Kumar Sen, Sunil Kardani, Rahul Trivedi, Rajesh Hadia, Cyril Sajan, Varunsingh Saggu, Sunil Baile, Kinjal Patel and Hemraj Singh Rajput

Abstract

The book chapter titled "Virtual Healing: Unveiling the Power of E-Consultation in Modern Healthcare" provides a comprehensive exploration of the evolution, current state, and future trends of E-Consultation. Beginning with an insightful examination of the historical perspective and technological advances shaping E-Consultation, the chapter delves into the landscape of virtual healthcare, emphasizing its current state, adoption, and integration in global healthcare systems. Extensive discussions on the benefits and challenges of E-Consultation shed light on how this virtual modality enhances patient accessibility, while also addressing critical issues such as the digital divide and privacy concerns. The chapter then shifts its focus to the crucial aspects of the patient-provider relationship in digital spaces, outlining strategies for building trust, effective communication, and maintaining a personal connection. Furthermore, the chapter explores the technological innovations propelling E-Consultation, including telehealth platforms, integration with Electronic Health Records (EHR), and the integration of Artificial Intelligence (AI). Each innovation is examined in detail, emphasizing their role in shaping the future of virtual healthcare delivery. The discussion extends to the regulatory framework, ethical considerations, and the delicate balance between innovation and patient safety. The chapter concludes with a forward-looking perspective, predicting future trends and innovations, highlighting the potential impact on traditional healthcare models, and issuing a call to action for healthcare professionals. Overall, the book chapter offers a comprehensive and insightful overview of E-Consultation, serving as a valuable resource for researchers, healthcare practitioners, and policymakers navigating the dynamic landscape of virtual healthcare.

Keywords: E-Consultation, virtual healthcare, telehealth, telemedicine,

technological innovations, Artificial Intelligence (AI), Electronic Health Records (EHR)

Introduction

E-Consultation, or electronic consultation, represents a transformative paradigm in the delivery of healthcare services, leveraging digital technologies to facilitate communication between patients and healthcare providers. In an era dominated by technological advancements, E-Consultation emerges as a pivotal component reshaping the landscape of modern healthcare. This chapter delves into the multifaceted dimensions of E-Consultation, exploring its definition, significance in contemporary healthcare, and providing a comprehensive overview of the topics covered.

Definition of e-consultation

E-Consultation refers to the electronic exchange of medical information and communication between patients and healthcare professionals, often facilitated through digital platforms or telehealth technologies. It encompasses a range of virtual interactions, including remote consultations, online diagnosis, and the exchange of medical records through secure digital channels (Hollander & Carr, 2020). This digital approach enables patients to seek medical advice without the need for in-person visits, offering a flexible and accessible alternative to traditional healthcare delivery models.

Significance in modern healthcare

The significance of E-Consultation in modern healthcare cannot be overstated, as it addresses several critical challenges facing the healthcare industry. With the increasing demand for healthcare services, especially in the context of global events such as the COVID-19 pandemic, E-Consultation provides a means to enhance healthcare accessibility, reduce geographical barriers, and optimize resource utilization (Wade *et al*., 2020). Moreover, it empowers patients by enabling timely access to medical expertise, fostering early intervention, and potentially reducing the burden on traditional healthcare infrastructure. The digital nature of E-Consultation also aligns with the growing preference for personalized and patient-centric care. Patients are increasingly seeking convenient ways to engage with healthcare providers, and E-Consultation serves as a conduit for fostering a more collaborative and responsive patient-provider relationship (Bashshur *et al*., 2016). Furthermore, it contributes to the efficient management of chronic conditions, preventive care, and the promotion of overall health and well-being.

Overview of the chapter

This chapter is structured to provide a comprehensive exploration of E-Consultation, covering various aspects to offer a nuanced understanding of its evolution, benefits, challenges, and future prospects. The following sections will delve into the historical development of E-Consultation, its current landscape, the benefits it offers to patients and healthcare systems, as well as the challenges and ethical considerations associated with its implementation. Additionally, the chapter will examine technological innovations driving E-Consultation, showcase real-world case studies, and discuss the regulatory frameworks shaping its practice. The exploration will conclude with insights into the future trends and potential impact on the traditional healthcare paradigm. In the subsequent sections, we will navigate through the historical evolution of E-Consultation, the current state of its implementation, and the transformative role it plays in reshaping patient-provider interactions.

Evolution of e-consultation

The evolution of E-Consultation can be traced through a historical lens, reflecting the convergence of healthcare needs and technological advancements. The concept of remotely accessing healthcare advice has roots in telemedicine, which emerged as early as the mid-20th century (Bashshur *et al*., 2016). However, it is in the past two decades that E-Consultation has witnessed significant growth, fueled by rapid advancements in digital technologies.

Historical perspective: The historical perspective of E-Consultation can be linked to the broader evolution of telemedicine. Early experiments with telemedicine date back to the 1950s when medical professionals explored the use of telecommunication technologies for transmitting radiological images (Bashshur *et al*., 2016). Over the following decades, telemedicine evolved, encompassing various forms of remote healthcare delivery, including telephonic consultations and video conferencing.

Technological advances shaping e-consultation: The shaping of E-Consultation is intricately tied to technological advances that have revolutionized the healthcare landscape. The advent of the internet, coupled with improvements in communication technologies and the widespread adoption of electronic health records (EHR), paved the way for the digitalization of healthcare services (Hollander & Carr, 2020). The rise of secure online platforms and the development of telehealth applications further accelerated the shift towards virtual consultations. Key Milestones in its Development: Several key milestones mark the development of E-Consultation as a mainstream component of modern healthcare. The early

2000s saw the emergence of pilot projects and experimental implementations of telehealth services in various regions (Wade *et al*., 2020). As technology continued to advance, dedicated platforms for E-Consultation were developed, providing secure and user-friendly interfaces for patients and healthcare providers. The COVID-19 pandemic acted as a catalyst, accelerating the adoption of E-Consultation globally. The imperative for social distancing and the strain on healthcare systems prompted a rapid shift towards virtual healthcare delivery models (Hollander & Carr, 2020). Governments and healthcare institutions invested in infrastructure and policy frameworks to support widespread E-Consultation services, solidifying its role as an integral part of the healthcare ecosystem. In summary, the evolution of E-Consultation reflects a journey from early telemedicine experiments to the current era of widespread digital healthcare access. Technological innovations and key milestones have propelled E-Consultation into a pivotal position, offering a flexible and accessible mode of healthcare delivery.

The landscape of e-consultation

The landscape of E-Consultation is dynamic and reflects the changing dynamics of healthcare delivery in the digital age. As technology continues to reshape the healthcare sector, E-Consultation has emerged as a transformative force, offering new possibilities for patient care and interaction with healthcare providers.

Current state of e-consultation services: The current state of E-Consultation services is characterized by a diverse range of platforms and applications that facilitate remote healthcare interactions. Telehealth companies and healthcare institutions have developed dedicated E-Consultation services that allow patients to connect with healthcare professionals through video calls, secure messaging, and virtual visits. Notable examples include platforms that provide specialized E-Consultation services in fields such as dermatology, mental health, and chronic disease management (Wade *et al*., 2020). The adoption of E-Consultation has accelerated, particularly in response to global events such as the COVID-19 pandemic. Governments and healthcare organizations worldwide have recognized the importance of E-Consultation in ensuring continuity of care while minimizing the risk of virus transmission. The flexibility and accessibility of virtual consultations have positioned E-Consultation as a valuable tool in maintaining healthcare services during crises (Hollander & Carr, 2020).

Adoption and integration in healthcare systems: The adoption of E-

Consultation is not only evident in standalone telehealth platforms but is also increasingly integrated into broader healthcare systems. Healthcare institutions are incorporating E-Consultation services into their comprehensive healthcare delivery models, creating seamless pathways for patients to transition between virtual and in-person care. This integration is facilitated by advancements in electronic health record (EHR) systems, enabling healthcare providers to access and update patient information in real-time, whether the encounter is virtual or physical (Wade *et al*., 2020). Furthermore, healthcare professionals are incorporating E-Consultation into their practice workflows, utilizing digital tools for remote monitoring, prescription management, and follow-up care. This integration enhances the efficiency of healthcare delivery and contributes to a more patient-centric approach.

Global trends and variances: The global landscape of E-Consultation exhibits trends and variances influenced by regional healthcare systems, technological infrastructure, and regulatory frameworks. Developed countries with robust digital infrastructure and widespread internet access often lead in the adoption of E-Consultation services, demonstrating higher rates of acceptance among both healthcare providers and patients. In contrast, developing regions may face challenges related to limited internet connectivity, digital literacy, and regulatory frameworks that may impact the widespread adoption of E-Consultation (Hollander & Carr, 2020). Government policies and reimbursement mechanisms also play a crucial role in shaping the adoption of E-Consultation services. In some regions, regulatory frameworks have been swiftly adapted to accommodate and encourage the use of telehealth and E-Consultation, while others may lag behind in creating a supportive environment. In conclusion, the landscape of E-Consultation is a rapidly evolving domain, characterized by a growing array of services, integration into healthcare systems, and global trends shaped by regional variations in infrastructure and regulation.

Benefits and challenges of e-consultation

E-Consultation, as a facet of telehealth, presents a spectrum of advantages and challenges, influencing both patients and healthcare systems. Understanding these dynamics is crucial for harnessing the full potential of virtual healthcare delivery.

Advantages for patients: One of the primary advantages of E-Consultation lies in the increased accessibility it affords to patients. Virtual consultations eliminate geographical barriers, enabling individuals in remote

or underserved areas to access healthcare expertise without the need for travel. This is particularly significant in addressing healthcare disparities and ensuring that individuals in rural or distant locations can receive timely medical advice (Wade *et al*., 2020). Additionally, E-Consultation enhances convenience for patients by offering flexible scheduling and reducing wait times. Patients can seek medical advice from the comfort of their homes, saving time and resources associated with traditional in-person visits. For individuals with chronic conditions, E-Consultation provides a continuous and convenient channel for monitoring and managing their health, contributing to improved outcomes and quality of life (Bashshur *et al*., 2016).

Enhancements in healthcare accessibility: The overarching impact of E-Consultation on healthcare accessibility extends beyond geographical considerations. It also addresses demographic and socioeconomic factors that can hinder traditional healthcare access. Vulnerable populations, such as the elderly or individuals with mobility limitations, benefit from the ease of virtual consultations, fostering inclusivity in healthcare delivery (Hollander & Carr, 2020). Furthermore, E-Consultation can play a pivotal role in preventive care and early intervention. By providing a mechanism for regular check-ins and monitoring, healthcare providers can identify potential health issues before they escalate, leading to proactive and cost-effective healthcare management (Wade *et al*., 2020).

Challenges and limitations in e-consultation: Despite its numerous advantages, E-Consultation is not without challenges and limitations. One significant challenge is the potential for a "digital divide," where individuals lacking access to technology or digital literacy may be excluded from the benefits of virtual healthcare. Addressing this challenge requires concerted efforts to bridge technological gaps and ensure equitable access to E-Consultation services (Hollander & Carr, 2020). Privacy and security concerns are another critical consideration. The exchange of sensitive health information in virtual spaces raises the importance of robust data protection measures to safeguard patient confidentiality. Healthcare providers and technology developers must adhere to stringent security standards to build and maintain trust in E-Consultation platforms (Bashshur *et al*., 2016). Moreover, E-Consultation may not be suitable for all medical conditions, and there are limitations in the scope of virtual interactions compared to in-person examinations. Certain diagnostic procedures, hands-on examinations, or emergencies may necessitate traditional face-to-face consultations. Striking the right balance between virtual and in-person care is essential to ensure comprehensive and effective healthcare delivery (Wade *et al*., 2020). In

conclusion, the benefits of E-Consultation in terms of improved accessibility and convenience for patients are evident. However, addressing challenges related to technology access, privacy, and the scope of virtual care is essential for its sustainable integration into modern healthcare systems.

Digital dialogues: The patient-provider relationship

The advent of E-Consultation brings forth the necessity for establishing robust digital dialogues within the patient-provider relationship. Building and maintaining trust, effective communication, and a personal connection in virtual spaces are critical components that underpin the success of E-Consultation, ensuring positive outcomes for both patients and healthcare providers.

Building trust in virtual spaces: Trust forms the cornerstone of any patient-provider relationship, and its establishment in virtual spaces is paramount. Patients need to feel confident in the security and confidentiality of their health information as it is exchanged digitally. E-Consultation platforms must adhere to stringent privacy standards, employing encryption and secure channels to safeguard patient data (Bashshur *et al*., 2016). Transparent communication about the security measures in place can contribute to building trust in the digital realm. Moreover, healthcare providers play a pivotal role in instilling trust during virtual consultations. Demonstrating empathy, professionalism, and a commitment to patient well-being through digital channels fosters a sense of reliability and confidence. Clear explanations about the virtual consultation process, diagnosis, and treatment plans contribute to a patient's understanding and trust in the healthcare provider (Hollander & Carr, 2020).

Effective communication strategies: Effective communication lies at the heart of successful virtual healthcare interactions. In digital dialogues, the absence of physical cues requires healthcare providers to employ clear and concise language, ensuring that patients comprehend medical information without the aid of non-verbal cues. Active listening becomes imperative, with providers encouraging patients to express their concerns and ensuring their questions are addressed comprehensively (Wade *et al*., 2020). The use of multimedia elements, such as visual aids or diagrams, can enhance communication by simplifying complex medical concepts. E-Consultation platforms that facilitate real-time sharing of information, such as test results or treatment plans, contribute to a more collaborative and informed patient-provider dialogue (Bashshur *et al*., 2016).

Maintaining a personal connection: Preserving a personal connection

in virtual spaces requires healthcare providers to go beyond the transactional aspects of medical consultations. Taking the time to inquire about patients' holistic well-being, understanding their lifestyle factors, and acknowledging their unique circumstances contribute to a more personalized healthcare experience (Wade *et al*., 2020). Furthermore, incorporating telehealth technologies that allow for face-to-face interactions can bridge the gap in the absence of physical presence. Video consultations enable providers to establish visual connections, reinforcing the human element of the patient-provider relationship. A personable and empathetic approach, even in virtual encounters, strengthens the emotional bond between patients and healthcare professionals (Hollander & Carr, 2020). In conclusion, digital dialogues in E-Consultation demand intentional efforts to build trust, employ effective communication strategies, and maintain a personal connection. Nurturing a positive patient-provider relationship in virtual spaces contributes to the overall success and acceptance of E-Consultation in modern healthcare.

Technological innovations in e-consultation

The landscape of E-Consultation is continually evolving, driven by technological innovations that enhance the delivery of virtual healthcare services. This section explores three pivotal technological advancements in E-Consultation: Telehealth Platforms and Tools, Integration with Electronic Health Records (EHR), and the integration of Artificial Intelligence (AI).

Telehealth platforms and tools: Telehealth platforms and tools represent the foundational infrastructure of E-Consultation, providing the digital space for patients and healthcare providers to connect remotely. These platforms encompass a range of communication tools, including video conferencing, secure messaging, and real-time chat functionalities. Notable examples include platforms like Zoom for Healthcare, Doxy.me, and other customized solutions developed by healthcare institutions (Bashshur *et al*., 2016). These platforms are designed to facilitate seamless interactions, ensuring high-quality audio-visual communication, secure data transmission, and user-friendly interfaces for both patients and healthcare providers. The user experience is a critical consideration, and advancements in telehealth platforms continue to focus on improving accessibility, ease of use, and overall user satisfaction (Hollander & Carr, 2020).

Integration with Electronic Health Records (EHR): Integration with Electronic Health Records (EHR) is a crucial technological advancement that enhances the efficiency and continuity of E-Consultation services. EHR integration allows healthcare providers to access patient medical history,

diagnostic reports, and treatment plans in real-time during virtual consultations. This seamless exchange of information ensures that healthcare providers have a comprehensive understanding of the patient's health status, enabling informed decision-making (Wade *et al*., 2020). The integration of EHR with E-Consultation platforms contributes to care coordination, as updates made during virtual consultations are automatically reflected in the patient's electronic health record. This interoperability between E-Consultation platforms and EHR systems streamlines administrative processes, reduces redundancy, and supports the delivery of more personalized and integrated healthcare services (Bashshur *et al*., 2016).

Artificial intelligence in e-consultation: Artificial Intelligence (AI) is increasingly becoming a transformative force in E-Consultation, offering innovative solutions to enhance diagnostic accuracy, treatment planning, and overall patient care. AI-powered applications analyze vast amounts of healthcare data to provide insights that support clinical decision-making. For example, AI algorithms can assist in interpreting medical imaging, identifying patterns in patient data, and even predicting potential health issues (Hollander & Carr, 2020). Chatbots and virtual assistants equipped with AI capabilities are being integrated into E-Consultation platforms to provide immediate responses to patient inquiries, schedule appointments, and offer personalized health information. AI-driven risk stratification tools help prioritize patient cases based on urgency, optimizing resource allocation in healthcare settings (Wade *et al*., 2020). While AI holds great promise in improving the efficiency and effectiveness of E-Consultation, ethical considerations and the need for ongoing validation and refinement of AI algorithms remain critical areas of focus for the healthcare industry (Bashshur *et al*., 2016). In conclusion, technological innovations in E-Consultation, including advanced telehealth platforms, integration with EHR, and the integration of AI, collectively contribute to the evolution of virtual healthcare delivery, offering enhanced capabilities and opportunities for improved patient care.

Case studies and success stories

The implementation of E-Consultation has yielded numerous case studies and success stories that highlight its efficacy in diverse healthcare settings. Examining these cases provides valuable insights into the tangible benefits, positive patient outcomes, and key lessons learned from successful E-Consultation initiatives.

Exemplary implementations of e-consultation: Several healthcare institutions globally have demonstrated exemplary implementations of E-Consultation, showcasing its versatility and adaptability across various specialties. For instance, the Veterans Health Administration (VHA) in the United States has successfully integrated telehealth services, including E-Consultation, to improve access to care for veterans, particularly those in remote areas (Darkins *et al*., 2008). The VHA's use of telehealth technologies has not only enhanced healthcare accessibility but also led to improved patient satisfaction and outcomes. In the private sector, specialty-focused E-Consultation platforms have emerged, such as teledermatology services that allow dermatologists to remotely diagnose and treat skin conditions. Studies have demonstrated the effectiveness of such platforms in reducing wait times for dermatological care and improving patient access to timely consultations (Snoswell *et al*., 2020).

Positive patient outcomes: Positive patient outcomes are a hallmark of successful E-Consultation implementations, contributing to improved healthcare quality and patient satisfaction. Research has indicated that virtual consultations can be as effective as traditional in-person visits for certain medical conditions, particularly in the realm of primary care and chronic disease management (Wade *et al*., 2020). Patients with chronic conditions, such as diabetes or hypertension, have experienced enhanced disease management through regular virtual check-ins, leading to better adherence to treatment plans and improved health outcomes. E-Consultation has also proven instrumental in mental health care. Remote access to mental health professionals through virtual platforms has facilitated timely interventions, reduced stigma associated with seeking mental health support, and contributed to positive mental health outcomes (Hollander & Carr, 2020).

Lessons learned from successful cases: While success stories abound, lessons learned from successful E-Consultation cases offer valuable insights for further refinement and widespread adoption. One key lesson is the importance of user-centric design in E-Consultation platforms. Platforms that prioritize user experience, simplicity, and accessibility tend to garner higher acceptance rates among both healthcare providers and patients (Wade *et al*., 2020). Effective implementation strategies involve comprehensive training for healthcare providers on the use of E-Consultation tools and protocols. Ensuring that providers are adept at navigating virtual consultations, maintaining patient engagement, and addressing technical issues contributes to the success of E-Consultation initiatives (Darkins *et al*., 2008). Additionally, successful cases emphasize the need for clear communication

with patients regarding the scope and limitations of virtual consultations. Establishing realistic expectations and ensuring patients are well-informed about the virtual care process fosters trust and acceptance of E-Consultation services (Snoswell *et al*., 2020). In conclusion, case studies and success stories in the realm of E-Consultation underscore its transformative impact on healthcare delivery. From specialty-focused implementations to improved patient outcomes, the lessons learned from successful cases provide a roadmap for the continued evolution and integration of E-Consultation into modern healthcare.

Regulatory framework and ethical considerations

As E-Consultation becomes an integral part of modern healthcare, navigating the regulatory landscape and adhering to ethical guidelines are paramount to ensure patient safety and the integrity of virtual healthcare delivery. This section explores the compliance and legal implications, ethical guidelines for E-Consultation, and the delicate balance between innovation and patient safety.

Compliance and Legal implications: The implementation of E-Consultation services necessitates strict adherence to regulatory frameworks to mitigate legal risks and ensure the delivery of high-quality care. Healthcare providers engaging in virtual consultations must comply with regional and national regulations governing telehealth practices. In the United States, for instance, adherence to the Health Insurance Portability and Accountability Act (HIPAA) is imperative to safeguard patient privacy and maintain the security of electronic health information (Hollander & Carr, 2020). Moreover, reimbursement policies and guidelines for E-Consultation services must align with existing healthcare reimbursement structures. Ensuring that telehealth services are recognized and reimbursed appropriately by insurance providers is crucial for the financial sustainability of E-Consultation initiatives (Wade *et al*., 2020).

Ethical guidelines for e-consultation: Ethical considerations form the foundation of virtual healthcare delivery, requiring healthcare providers to uphold principles of professionalism, confidentiality, and patient-centered care. The American Medical Association (AMA) and other medical associations globally have established ethical guidelines specific to telemedicine and E-Consultation. These guidelines emphasize the importance of informed consent, maintaining patient confidentiality, and establishing clear communication about the limitations of virtual consultations (Bashshur *et al*., 2016). Furthermore, the principles of non-discrimination and equity

must be preserved in E-Consultation services. Ensuring that virtual healthcare is accessible to individuals of diverse backgrounds, regardless of socioeconomic status or technological literacy, aligns with ethical imperatives of healthcare equity (Hollander & Carr, 2020).

Balancing innovation with patient safety: In the pursuit of advancing E-Consultation technologies, a delicate balance must be struck between innovation and patient safety. Rapid technological advancements, including the integration of artificial intelligence, bring forth new possibilities for improving diagnostic accuracy and treatment planning. However, the deployment of these technologies requires rigorous testing, validation, and ongoing monitoring to ensure their safety and effectiveness (Wade *et al*., 2020). Ethical considerations extend to the responsible integration of emerging technologies, safeguarding against potential biases in AI algorithms and ensuring that they enhance, rather than compromise, the quality of care. Additionally, healthcare providers must remain vigilant in detecting and addressing issues related to cybersecurity to protect patient data and maintain the integrity of E-Consultation platforms (Bashshur *et al*., 2016). In conclusion, the regulatory framework and ethical considerations surrounding E-Consultation are critical components of its successful integration into modern healthcare. Compliance with legal requirements, adherence to ethical guidelines, and a cautious approach to innovation collectively contribute to a secure, ethical, and patient-centered virtual healthcare environment.

Future trends and innovations

The landscape of E-Consultation is poised for continuous evolution, driven by emerging technologies that promise to reshape the future of virtual healthcare delivery. This section explores the current trends and potential innovations that are likely to shape the trajectory of E-Consultation, offering insights into the predicted future of virtual healthcare.

Emerging technologies in e-consultation: Several emerging technologies are set to play a transformative role in E-Consultation. One of the notable advancements is the integration of virtual reality (VR) and augmented reality (AR) technologies. VR and AR can enhance the virtual healthcare experience by providing immersive environments for medical simulations, surgical planning, and patient education. These technologies have the potential to simulate physical presence, allowing healthcare providers to conduct more comprehensive virtual examinations and procedures (Hollander & Carr, 2020). The Internet of Things (IoT) is another emerging technology that holds promise for E-Consultation. Wearable devices and remote

monitoring technologies can continuously collect and transmit real-time health data to healthcare providers. This continuous stream of data enables proactive healthcare interventions, personalized treatment plans, and improved management of chronic conditions, contributing to more effective virtual care (Bashshur *et al*., 2016).

Predictions for the future of virtual healthcare: Predicting the future of virtual healthcare involves envisioning a healthcare landscape where E-Consultation becomes an integral and seamlessly integrated component of patient care. The continued refinement of telehealth platforms and the widespread adoption of interoperable electronic health records (EHR) are expected to create a more cohesive and interconnected virtual healthcare ecosystem. This interoperability will facilitate the seamless exchange of patient information, ensuring that healthcare providers have a comprehensive view of the patient's health history during virtual consultations (Wade *et al*., 2020). The future of E-Consultation also includes the expanded use of artificial intelligence (AI) in healthcare delivery. AI algorithms will likely become more sophisticated in diagnosing medical conditions, predicting patient outcomes, and supporting clinical decision-making. Chatbots and virtual health assistants powered by AI will offer more personalized and immediate responses to patient inquiries, contributing to enhanced patient engagement and satisfaction (Hollander & Carr, 2020).

Potential impact on traditional healthcare models: The predicted future of E-Consultation implies a substantial impact on traditional healthcare models. Virtual healthcare has the potential to redefine the patient-provider relationship, offering a more patient-centric and accessible approach to healthcare delivery. The increased adoption of E-Consultation may lead to a shift in the utilization of healthcare resources, with a greater emphasis on virtual care as a primary mode of interaction. Additionally, the potential impact extends to the restructuring of healthcare delivery settings. The increased prevalence of virtual healthcare may reduce the reliance on traditional brick-and-mortar healthcare facilities, leading to the development of hybrid models that blend virtual and in-person care. This transformation may contribute to more efficient resource allocation, reduced healthcare costs, and improved healthcare accessibility on a global scale (Bashshur *et al*., 2016). In conclusion, the future of E-Consultation is intricately tied to the adoption of emerging technologies, the refinement of virtual healthcare models, and the potential reconfiguration of traditional healthcare frameworks. As these trends unfold, the vision of a more connected, innovative, and patient-centered healthcare future comes into focus.

Conclusion

In conclusion, E-Consultation represents a transformative shift in modern healthcare, marked by technological innovations, ethical considerations, and a commitment to patient-centric care. Throughout our exploration, key themes have emerged, emphasizing the advantages of E-Consultation in enhancing accessibility, improving patient outcomes, and reshaping traditional healthcare models. The future of virtual healthcare holds promise with emerging technologies like VR, AR, IoT, and AI, poised to redefine the patient-provider relationship and healthcare delivery. A call to action for healthcare professionals involves embracing these innovations, staying informed on regulatory developments, and actively participating in the ethical evolution of virtual healthcare. As we navigate the future, the vision for E-Consultation is not just about technology; it's about creating a healthcare landscape that prioritizes inclusivity, quality, and patient empowerment—a vision that holds the potential to revolutionize the way we approach and deliver healthcare services globally.

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Chapter - 2
Artificial Intelligence Useful for Kids

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Chapter - 2

Artificial Intelligence Useful for Kids

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Abstract

Early childhood education (ECE) has faced several fundamental challenges as a result of artificial intelligence (AI) education. These challenges include the following: (1) why AI is appropriate and necessary for learning in the early years; (2) what is the subset of important AI ideas and concepts that children can learn; and (3) how to engage children in meaningful experiences that enable them to acquire these fundamental AI concepts. The main factors to take into account while creating an AI curriculum for young children are covered in this paper from the ECE area.

The fundamentals of AI that can be discussed with young children are as follows: With a lot of data, AI algorithms can be continuously trained to recognise patterns, anticipate outcomes, and suggest courses of action—albeit with some restrictions. Young children's exploration with AI devices should be facilitated by an embodied, culturally relevant approach, based on the theoretical concepts of learning-by-making and pedagogy-as-relational. In order to illustrate this pedagogical model and provide educators an explanation of how to give children culturally appropriate inquiry chances to interact with and comprehend AI technologies, an exemplary curriculum called "AI for Kids" is finally introduced. The combination of knowledge about the "Why," "What" and "How" of AI education for young children inspires innovative approaches to getting kids interested in STEM subjects and helping them understand the digital environment.

How AI (Artificial Intelligence) is revolutionizing the world. From finance to healthcare, AI is nothing new-it has been around for decades and is now used in almost every aspect of our lives.

But the recent launch of AI tools has thrust this technology into the spotlight, leading to a growing awareness of its potential implications-especially for younger generations and those who will come after them.

It is essential that we understand exactly how AI will affect the lives of children-both now and in the future. With technology moving faster than ever before, it is essential that we consider the implications of machine learning and artificial intelligence and how AI literacy needs to be taught from an early age.

Artificial intelligence (AI) is nothing new; it has been employed in practically every facet of our life for decades, from healthcare to banking.

However, the recent release of AI tools has brought this technology to light and raised awareness of its possible consequences, particularly for the next generation and those born before them.

It is critical that we comprehend the precise ways in which artificial intelligence will impact children's lives, both now and in the future. Since technology is advancing more quickly than ever, it is critical that we take artificial intelligence and machine learning into consideration.

Keywords: Artificial intelligence, health care & children

Introduction

The simulation of human intelligence processes by machines, particularly computer systems, is known as artificial intelligence (AI). Expert systems, natural language processing, speech recognition, and machine vision are a few specific uses of AI.

AI-enabled applications across a wide range of industries, including robotics, social media, healthcare, automotive, and education. Moreover, it is seen that AI education has the ability to significantly enhance children's learning because it may incorporate knowledge from multiple disciplines and technologies at the same time [2]. Prior research demonstrates that artificial intelligence-powered interfaces facilitate the usage of child-computer interactions like gesture, touch, and speech to access digital material and services.

Artificial intelligence (AI) technologies are radically altering the world and influencing children in the present and the future. AI technologies are already being used by youth in a variety of contexts, including toys, virtual assistants, video games, and adaptive learning applications. Children get recommendations from algorithms about what videos to watch next, what news to read, what music to listen to, and who to make friends.

It should be also noted that AI is not only useful for development of child, in child and adolescent psychiatry, there are areas where wearable technology advancements can supplement the present in-person physician

examinations to individualize the diagnosis and treatment of psychiatric problems. It is also under investigation to use artificial intelligence in detecting dental caries in children and approaches for pediatric rehabilitation that focus on getting children and young adults with disabilities or other medical issues involved in worthwhile activities.

Necessity of AI learning in children

Every person should study about artificial intelligence since it will someday impact more areas of human existence than just the computer industry. Nowadays, AI is being used in a wide range of fields (such as business, research, art, and education) to boost productivity as well as enhance user experience. In a becoming intelligent society, understanding and appreciating the fundamentals of artificial intelligence (AI) and utilizing AI applications is an inherent component of digital literacy for all citizens.

Creating educational activities that leverage age-appropriate tools like AI-interfaced robots and applications to cascade young children's comprehension of artificial intelligence is crucial. Exposing kids to concepts of machine learning. It should be seen that all children and their families, particularly those from less advantaged homes, have access to information literacy programmes as well as the usage of technological advances, especially AI technologies, as we are entering an era powered by artificial intelligence.

Early AI learning necessitates efforts towards sustainable development that, in the long term, can lead to digital inclusion and equity and reduce the digital divide in early childhood education. However, we inhabit a digital world that is evolving quickly and where AI is now a constant in every aspect of our lives, there is a dearth of study on the integration of AI in early childhood education. It is still uncommon to know how to incorporate AI literacy into early childhood curricula.

Integration of literacy in artificial intelligence in younger generations

The common understanding of literacy was the capacity for reading and writing. The advent of the knowledge-based society in the current digital age suggests that all citizens need to qualify as "digitally literate" and acquire fundamental skills for the purpose to have more access to equitable possibilities at work. In addition to learning ways to distinguish amongst ethically acceptable and unethical actions, students also need to learn how to use AI technologies responsibly.

It is evident that there are four components that make up AI literacy: knowledge and comprehension of AI, application and usage of AI,

assessment and development of AI, and ethical behavior related to AI. In a study, young children were taught three artificial intelligence concepts comprising knowledge-based systems, supervised machine learning, and generative AI, and then evaluated and analyzed their AI literacy and their behaviour.

In another study, a detailed examination was done using a detailed framework as follows-

Elements	Descriptions	Examples
AI concepts	Technical and conceptual comprehension of AI's fundamental operations	Categorization, forecasting, and production
AI practices	The methods and approaches applied while utilizing AI	Evaluation, validation, and training
AI perspectives	Inclinations and attitudes used when problem-solving	Collaborating cooperatively to identify solutions and being aware of the power of technology as an aid for resolving issues

Leveraging a publicly accessible agent/system to create an AI literacy course for young kids is a big and influential practical application. Quick, Draw! and Teachable Machine are a few of these publicly available intelligent agents that can be utilized to create AI literacy curricula for use in early childhood education settings.

Implementation of AI literacy

Young children should learn AI literacy through a situationally oriented curriculum. Any material that is to be learnt should be connected to a specific context that is pertinent to the experiences of the learners, according to this embodied approach. According to the theory of situative embodiment, a curriculum should have a plot that gives students purposeful objective, anticipated behaviors, and a setting in which to study. Crucially, this type of embodiment will offer a contextual framework that helps students understand the relevance of the course material.

For embedding AI in a child’s learning, the following is necessary-

1. Children should have a welcoming and socially linked learning environment in addition to the AI engagement. Educators can help kids accomplish this by encouraging sharing, collaboration, cooperative instruction, and collaborative educational strategies.

2. The AI assignment must be connected to the children's past knowledge, abilities, or experiences. Before delving further into more complex tasks or projects, children might be presented to certain cultural activities or equipment that they are already familiar with.
3. Educators should incorporate educational activities (such as design challenges) that call for the use of higher-order thinking abilities in inquiry projects into the primary learning endeavor. The task ought to be problem solving, action-oriented, and applicable to a few real-world situations.
4. Children's problem-solving abilities and real-world contexts should be taken into consideration while assessing their achievements and advancement in AI education. Authentic assessment tasks, portfolios, artefacts, self-evaluation, and prompt feedback are a few of these methods of evaluation.

Examples on imperativeness of Artificial intelligence in children

1. A research team in Hong Kong created the Programme dubbed "AI for Kids". This curriculum seeks to show how AI-powered technology are used in everyday life and to give kids the opportunity to learn about AI through an embodied, project-based approach. The project's main goal is environmental preservation, particularly that of the ocean, which speaks to the educational interests of Hong Kong youngsters on a cultural level. Children are also given an assignment in the "AI for Kids" curriculum that asks them to sketch AI using their imaginations. By seeing how imaginatively the kids approach the problem, teachers can gauge how imaginative the kids are with AI.
2. Throughout an individual's lifetime, mental health disorders are a primary source of medical disability. Due to difficulty in diagnosis and lack of precision medical methods, this burden is especially high in children and adolescents. Nonetheless, it is encouraging that wearable technology such as smart watches is becoming widely used. This technology makes it possible for artificial intelligence programmes to remotely diagnose and treat psychological illnesses in kids and teenagers. Although research in this field is very limited, it is seen that individual with ADHD and autism spectrum disorder are easy to diagnose with aid of this technology.

The impact of artificial intelligence on children & future generations

AI technology has the potential to revolutionize practically every aspect of life, from healthcare to education. It is easy to see how the concept of AI can cause concern – but if we look beyond the implications of machines taking over jobs, we can see how AI can be used to improve our lives in countless positive ways.

Here are a few of the top benefits that AI could bring to children and future generations:

1. Improved educational experiences

Education requires constant tracking of metrics and creation of content to meet the needs of students. AI can automate this process, enabling more efficient data analysis & content generation. This can help ensure that the educational experience is tailored to each student's needs, thereby providing them with a better chance of success.

For example, generative AI can be used to build chat bots that can provide students with tailored advice in real time while they are working on their tasks. AI can also be used to track a student's development and provide recommendations or interventions to help them meet their learning objectives. Additionally, content can be created and given to students according to their particular requirements and interests, enabling a customised learning environment.

2. Improved healthcare outcome

AI can be used to analyse large amounts of patient data to identify potential medical issues that could go undetected by traditional methods. This can help doctors diagnose and treat their patients more accurately and quickly, and AI-powered bots can identify potential risks and provide healthcare advice. For example, AI-based chatbot systems can answer patient questions, freeing up time for doctors and nurses to focus on more complex medical tasks.

For children in particular, AI systems can provide a more personalized healthcare experience. For instance, AI-based systems can use facial recognition and voice recognition to identify individuals and provide tailored advice or medical treatments.

3. More secure digital & physical environments

Data security continues to be a growing concern for all of us, and AI systems take online security and protection a step further. AI can help detect

malicious activity on the internet and identify potential threats, thereby providing a more secure environment for children online. AI-based systems can also monitor our homes and ensure our children are safe when we are away & when combined with the Internet of Things (IoT) technology, AI systems can learn our habits and provide automated responses to potential security issues.

4. Expanded access to resources

Children need the ability to access resources to learn and grow, but unfortunately, not all children have access to the same level of education or resources. However, AI-based systems can help bridge this gap by providing resources and teaching tools tailored to each child's needs.

AI-based systems can also provide access to vast libraries of educational material, from books and videos to interactive simulations and games. This would enable children all over the world to gain access to resources they would otherwise never have had access to.

5. Greater opportunities for career and skill development

AI is not going to replace humans in the workforce, after all, AI systems are designed to enhance and supplement the work we do. But AI can help make specific tasks more efficient or provide us with more opportunities for skill development.

For instance, children and teens could use AI-based systems to practice tests and interviews, preparing them better for any career path they choose. College students could also use AI-based systems to help them gain access to larger opportunities for jobs and internships.

By closing the gap between education and the modern workforce, AI-based systems can help children and teens gain the skills they need for a successful future.

The negatives of AI and our children's future

The benefits that AI promises are incredibly exciting, but as with any technology, there are potential risks and drawbacks.

1. A reduced amount of human interaction

Children and teens currently face an epidemic of social isolation, and AI-based systems could further reduce the time they spend interacting with others. If children become too reliant on AI-based systems, it could cause them to miss out on the critical human connection essential for their development.

One example is a move toward engaging with AI tools for therapy and counselling. Rather than schedule appointments with trained mental health professionals, children could instead use AI-based services.

While this could create greater access to life-saving mental health services, it could also reduce children's personal contact with other people & trusting an AI output without knowing its underlying training processes threatens children's autonomy and identity formation.

2. The creation of a larger "Digital divide"

Due to the costs associated with AI-based systems, they could create an even greater digital divide between those who have access to such resources and those who don't.

With AI-based systems being cheaper than hiring teachers and counsellors, they could also replace human employees entirely. This would lead to fewer job opportunities for those who are already disadvantaged – or reduce the amount of meaningful work they can find.

3. Diminished creativity and problem-solving skills

AI-based systems can take over many tasks that humans would have once done, such as testing, grading, and giving feedback on student projects. While this promises to free up more time for educators to focus on in-depth, meaningful instruction, it may also reduce the creative thinking and problem-solving abilities children develop.

Too much automation in education could lead to students lacking creativity or critical thinking skills. For instance, plagiarism has become an issue with AI-based systems. It's not only crucial for educators to teach children the importance of being creative and staying away from plagiarism, but it's also beneficial for them to learn how to think critically and solve problems independently.

4. Ethical implications of data collection

AI is powered by data collection. Therefore, naturally, the more we rely on AI-based systems, the more data we will collect about our children. This brings up numerous ethical and privacy concerns that need to be addressed to use AI responsibly.

Data collection can also potentially harm children by creating biased results against certain demographic groups.

For example, facial recognition algorithms might not work as well on darker skin tones, and generative programs that utilize online text may

capture hate speech or offensive stereotypes. If children and teens cannot recognize the potential biases of AI, they may be at risk of developing negative thinking patterns.

5. A reduction in critical thinking

Possibly one of the most significant issues with AI is the potential for a decrease in critical thinking skills. As AI becomes more prevalent, it's crucial to ensure that children are not relying solely on automation and computers to solve their problems.

Instead, they should be encouraged to think independently and come up with creative solutions of their own. They should also be taught to evaluate data and information carefully to identify any potential biases in the results they receive.

By fostering a culture of independent thinking and critical assessment, children will be better prepared for life with AI in the future.

Conclusion

The topic of early childhood digital literacy is informed by this paper, which focuses on equipping young children with age-appropriate tools for introducing machine learning models and effective pedagogical approaches. We are able to reconsider the significance of concrete AI-related learning experiences in early childhood education thanks to the concept of culturally sensitive initiatives. Furthermore, in a variety of early childhood education contexts, haptic education provides a pedagogical strategy for fostering AI literacy.

In addition to increasing children's exposure to AI technology, this pedagogical approach and its use in curriculum design and implementation will improve their ability to reason thoughtfully and nuancedly about AI, an inclusive mind tool that is crucial for thriving in the modern age of technology. Child development experts and pedagogical experts will find support for the initiative of digital equity, which advocates for AI literacy for all, including young children. Learning AI, will also offer the fundamentals for creating and executing AI curricula for young children in formal (like preschools) and informal (like homes, museums, and libraries) learning environments. Through integrated educational opportunities, preschoolers will be emboldened and given the opportunity to gain AI literacy, thereby preparing them for a cognitive era

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Chapter - 3
Role of Artificial Intelligence in Drug Delivery: A Review

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Chapter - 3

Role of Artificial Intelligence in Drug Delivery: A Review

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Abstract

Advancements in artificial intelligence (AI) have catalyzed a transformative era in healthcare, with profound implications for drug delivery systems. This review paper explores the pivotal role of AI in revolutionizing drug delivery, offering a comprehensive overview of the synergistic integration of AI technologies into drug development, formulation, administration, and monitoring. AI-powered drug discovery has emerged as a promising approach to accelerate the identification of novel drug candidates, predict their pharmacokinetics, and optimize their chemical properties. Moreover, AI-driven formulation design enables the creation of tailored drug delivery systems, enhancing drug solubility, stability, and bioavailability. Precision medicine benefits from AI algorithms that personalize drug delivery, ensuring optimal therapeutic outcomes while minimizing side effects. In drug administration, AI-driven devices, such as smart injectors and implantable sensors, provide real-time monitoring and control, allowing for precise dosage adjustments. Furthermore, AI-based algorithms optimize drug dosing schedules, minimizing patient burden and improving treatment adherence. The role of AI extends to the monitoring of drug efficacy and safety. AI-driven data analysis and predictive modeling enable the early detection of adverse events and treatment response assessment, facilitating timely interventions and reducing healthcare costs. This review paper critically examines the current state of AI applications in drug delivery systems, highlighting the challenges and opportunities associated with their implementation. Ethical and regulatory considerations are also discussed to ensure responsible and safe AI integration in healthcare.

Keywords: Artificial intelligence (AI), drug delivery systems, drug discovery, precision medicine, formulation design, personalized treatment, etc.

Introduction

Background of the study

The background of the study on the role of AI in drug delivery systems is rooted in the rapidly evolving landscape of healthcare and pharmaceuticals (Vora *et al.*, 2023a). Traditional drug development and delivery processes are often time-consuming, costly, and inefficient, leading to delayed access to life-saving medications and suboptimal treatment outcomes. In this context, AI technologies have emerged as a powerful force for innovation and improvement in the field. (Paul *et al.*, 2021).

1) **Drug development challenges:** The pharmaceutical industry has long grappled with challenges in drug discovery and development. These challenges include the identification of suitable drug candidates, understanding complex pharmacokinetics, and optimizing rug formulations for efficacy and safety. (Vora *et al.*, 2023b) These hurdles contribute to the high attrition rates and lengthy timelines associated with bringing new drugs to market. (Munos, 2009)
2) **Personalized medicine paradigm:** Healthcare is increasingly moving towards personalized medicine, where treatments are tailored to individual patients based on their unique genetic makeup, lifestyle, and medical history. (Mak *et al.*, 2019) Achieving this level of customization in drug delivery requires sophisticated technologies capable of processing vast amounts of patient data and adapting treatment regimens accordingly.
3) **AI advancements:** Over the past decade, AI has made significant strides in various domains, including natural language processing, computer vision, and machine learning. (Hosny *et al.*, 2018) These advancements have enabled the development of AI algorithms capable of analysing complex biological data, predicting drug interactions, and optimizing drug formulations, among other applications.
4) **Healthcare technology integration:** The integration of AI in healthcare is becoming increasingly common, with electronic health records (EHRs), wearable devices, and telemedicine platforms being prime examples. (Raikar *et al.*, 2023a) These technologies create opportunities for real-time monitoring, data collection, and decision support, all of which are crucial for effective drug delivery systems. (Naik *et al.*, 2022a)

5) **Ethical and regulatory concerns:** The introduction of AI into healthcare raises ethical and regulatory considerations. Ensuring patient privacy, data security, and the responsible use of AI algorithms are paramount concerns. (De Almeida *et al.*, 2021) Moreover, regulatory agencies worldwide are working to establish guidelines and frameworks for AI-driven healthcare applications to ensure safety and efficacy. (Bajwa *et al.*, 2021)
6) **Patient-centric care:** The ultimate goal of AI in drug delivery is to enhance patient care by improving treatment outcomes, reducing adverse events, and increasing treatment adherence. Patient-centric care is becoming a central tenet of modern healthcare, and AI has the potential to make this vision a reality. (Blanco-González *et al.*, 2023)

In light of these developments and challenges, the study seeks to provide a comprehensive examination of how AI is being harnessed to address longstanding issues in drug delivery systems. It aims to explore the potential benefits, ethical considerations, and regulatory implications of AI integration, ultimately contributing to a deeper understanding of the transformative role AI plays in the pharmaceutical and healthcare industries. (Yang *et al.*, 2019)

Artificial intelligence evolution

In 1956, John McCarthy proposed a phrase "Artificial Intelligence" and this concept given by Talus and Pygmalion. (Liebman, 2022)

Artificial intelligence refers to machines, particularly computer systems, simulating human intelligence processes. AI is a component of technology, including machine learning, and relies on specialized hardware and software for creating and training machine learning algorithms. AI systems function by processing large sets of labeled training data, identifying patterns and correlations, and using these patterns to predict future outcomes. (Haleem *et al.*, 2022)

AI primarily focuses on cognitive skills such as learning, reasoning, self-correction, and creativity. It's a field that combines computer science with robust datasets to facilitate problem-solving. Within AI, you'll often encounter sub-fields like machine learning and deep learning, which are closely associated with it. AI empowers machines to replicate or enhance human-like capabilities, and its integration into daily life is steadily growing, with companies across various industries investing in it. (Rahman, 2020)

The foundation of artificial intelligence lies in the idea that human intelligence can be defined in a manner that machines can emulate, performing

tasks ranging from simple to highly complex. The objectives of AI encompass mirroring human cognitive processes, with researchers and developers rapidly progressing in emulating activities like learning, reasoning, and perception, often with well-defined outcomes in mind. An ideal characteristic of artificial intelligence is its ability to reason and make decisions that optimize the chances of achieving specific goals. (De Bruyn *et al.*, 2020)

The evolution of artificial intelligence (AI) in the field of drug delivery systems has been marked by significant advancements and milestones over the years. This evolution has fundamentally transformed the way pharmaceuticals are developed, formulated, administered, and monitored. (Bender *et al.*, 2021) Here is a timeline highlighting key developments in the AI-driven evolution of drug delivery systems:

1) **Early applications (2000s):** In the early 2000s, AI was primarily applied to drug discovery, where computational methods were used to screen large chemical libraries for potential drug candidates. Virtual screening and molecular modelling played a crucial role in identifying lead compounds for further development. (Han *et al.*, 2023)
2) **Predictive modelling (2010s):** During the 2010s, AI-powered predictive modelling gained prominence. Machine learning algorithms, such as deep learning and random forests, were employed to predict drug-protein interactions, pharmacokinetics, and toxicity profiles. This allowed for faster and more accurate drug candidate selection. (Dara *et al.*, 2022)
3) **Formulation optimization (2010s):** AI-driven formulation optimization became a focus in the pharmaceutical industry. Machine learning algorithms were used to design drug formulations with improved solubility, stability, and bioavailability, reducing formulation development timelines and costs. (Lu *et al.*, 2023)
4) **Personalized medicine (2010s-Present):** The concept of personalized medicine gained traction, with AI algorithms being used to analyse patient data and genetic information to tailor drug delivery regimens to individual patients. (Schork, 2019) This approach aimed to optimize treatment efficacy while minimizing side effects.
5) **Smart drug delivery devices (2010s-Present):** The development of smart drug delivery devices equipped with AI capabilities became a significant trend. These devices, such as insulin pumps and inhalers, use AI to monitor patient adherence, provide real-time feedback, and

adjust dosages automatically for precise drug delivery. (Raikar *et al.*, 2023b)

6) **Real-time monitoring and feedback (2010s-Present):** AI-powered sensors and wearable devices allow for real-time monitoring of patient vital signs, biomarkers, and medication levels. These devices provide healthcare professionals with valuable data for adjusting drug regimens and making timely interventions. (Wang *et al.*, 2023)
7) **AI in clinical trials (2010s-Present):** AI is increasingly being used to optimize the design and execution of clinical trials. Predictive analytics and data-driven insights help identify suitable patient populations, predict trial outcomes, and streamline the drug development process. (Weissler *et al.*, 2021)
8) **Ethical and regulatory considerations (2010s-Present):** The integration of AI in drug delivery systems has raised ethical and regulatory concerns related to patient privacy, data security, and algorithm transparency. Regulatory agencies have been working to establish guidelines to ensure the safe and responsible use of AI in healthcare. (Gerke *et al.*, 2020)
9) **Future prospects (2020s and Beyond):** The future of AI in drug delivery systems holds the promise of even more advanced applications. This includes the use of AI-driven nanotechnology for targeted drug delivery, AI-enabled robotic systems for precise drug administration, and further advancements in personalized medicine. (Das *et al.*, 2023a)

The evolution of AI in drug delivery systems reflects a transformative shift in the pharmaceutical and healthcare industries, with AI-driven innovations poised to improve treatment outcomes, reduce costs, and enhance patient care. As AI technologies continue to evolve, the field of drug delivery will likely witness further breakthroughs, ultimately leading to more effective and patient-centric healthcare solutions. (Ahuja, 2019)

Role of AI in drug delivery systems

AI-assisted formulation design has become increasingly popular in recent years for improving drug distribution and bioavailability. Machine learning algorithms are employed to optimize medication formulations by analysing vast amounts of data on drug properties like solubility, permeability, and stability. This approach can enhance drug bioavailability, which refers to the amount of a drug that reaches the bloodstream and its target. Many drugs face low bioavailability due to issues like poor solubility or fast metabolism, but

AI can improve their effectiveness. Additionally, AI can enhance drug delivery by optimizing formulations, potentially enabling new delivery methods such as targeted drug delivery. AI-assisted formulation design has the potential to expedite the development of new medications by reducing the number of tests needed to find the best formulation. (Murray *et al.*, 2023)

In the realm of drug administration, various types of artificial neural networks, including deep or neural networks, are utilized. A key strategy to enhance drug administration success involves targeting the proteins involved in drug distribution. Artificial intelligence (AI) is the capability of a machine to mimic human cognitive functions. Machine learning, a component of AI, involves using algorithms and pattern recognition to identify trends in data for decision-making. Machine learning encompasses the ability to learn patterns in input data without explicit instructions, including numerical regressions and categorization. This capability enables the creation of meaningful outputs from given inputs. Machine learning techniques can analyse large datasets of drug activity in the body and predict drug reactions. (Askr *et al.*, 2023)

Artificial neural networks (ANNs), which resemble the structure of the human brain, are frequently used for deep learning in machine learning. ANNs outperform traditional machine learning methods due to their computational and predictive capabilities. They possess characteristics like discovery, tolerance, learning, non-linearity, resilience, and handling ambiguous and imperfect information. ANNs can address complex tasks such as optimization, pattern classification, clustering, and pattern recognition. They come in various types, including associating networks, feature-extracting networks, and non-adaptive networks. (I. Singh *et al.*, 2023)

Robotics is another critical field related to AI, requiring advanced capabilities in surveillance, reporting, item handling, navigation, mapping, localization, and motion planning. Robots have proven significantly more efficient than humans in tasks like manufacturing oral and injectable medications, including hazardous chemotherapy drugs. AI-driven models have been successful in developing pharmacological dosage forms, especially tailored drug delivery systems for specific patient populations. Machine learning algorithms are employed to enhance drug compositions using large datasets of drug behaviour. Deep learning algorithms like recurrent neural networks and convolutional neural networks have been effectively utilized in pharmaceutical sciences for various formulation-related tasks. (Gyles, 2019).

In summary, AI-assisted formulation design, drug administration, and robotics are interconnected fields where AI and machine learning play pivotal

roles in enhancing drug development, administration, and manufacturing processes. These technologies have the potential to revolutionize the pharmaceutical industry. (Kolluri *et al.*, 2022)

AI in Nano-medicines

The modification of nanoparticles for drug delivery has become crucial in therapeutics and diagnostics due to improved efficacy and therapy. Combining nanotechnology with AI offers solutions to various formulation development challenges. For instance, AI was used to computationally design a methotrexate Nano suspension by analysing drug molecule interactions and variables influencing formulation stability. AI can also assist in studying drug-dendrimer interactions and assessing drug encapsulation within dendrimers using techniques like coarse-grained simulation and chemical calculations. Furthermore, tools like LAMMPS and GROMACS 4 aid in investigating how surface chemistry affects nanoparticle uptake into cells. AI has contributed to the development of silicasomes, which consist of tumour-penetrating peptides and irinotecan-loaded multifunctional mesoporous silica nanoparticles. This innovation significantly improved treatment outcomes and overall survival by enhancing silicasome uptake through transcytosis induced by the tumour-penetrating peptide iRGD. (A. P. Singh *et al.*, 2019)

Nano medicine researchers can leverage AI's capabilities in data analysis, pattern recognition, and optimization to expedite the development of innovative nanoscale interventions, enhance diagnostics, improve drug delivery, and advance personalized medicine. The integration of AI into Nano medicine has the potential to transform healthcare by enabling precise and targeted therapeutic approaches at the nanoscale. (Adir *et al.*, 2020)

One crucial application is the use of nanoparticles for targeted drug delivery, imaging, and sensing. AI algorithms can assist in designing and fine-tuning nanoparticles by predicting their physical and chemical properties, stability, and effectiveness, allowing researchers to create nanoparticles tailored for specific purposes, such as drug delivery. (Haleem *et al.*, 2023)

Nanomedicines are particularly valuable for treating cancer patients, as they enable drug selection, dosage optimization, and the use of stimuli-responsive materials. Deep learning algorithms have shown impressive accuracy in melanoma care and diagnostics. (P.M. Giri *et al.*, 2023)

AI algorithms can also model how nanoscale materials interact within biological systems, aiding in predicting nanoparticle behaviour, drug release rates, and potential toxicity. This facilitates the development of safe and efficient nano-medicine formulations. (Das *et al.*, 2023b)

In addition to drug delivery, AI plays a role in nano sensors and biosensors for real-time monitoring of biomarkers, drug levels, and disease progression. These sensors offer continuous feedback to healthcare providers, allowing for personalized treatment adjustments. (Manickam *et al.*, 2022)

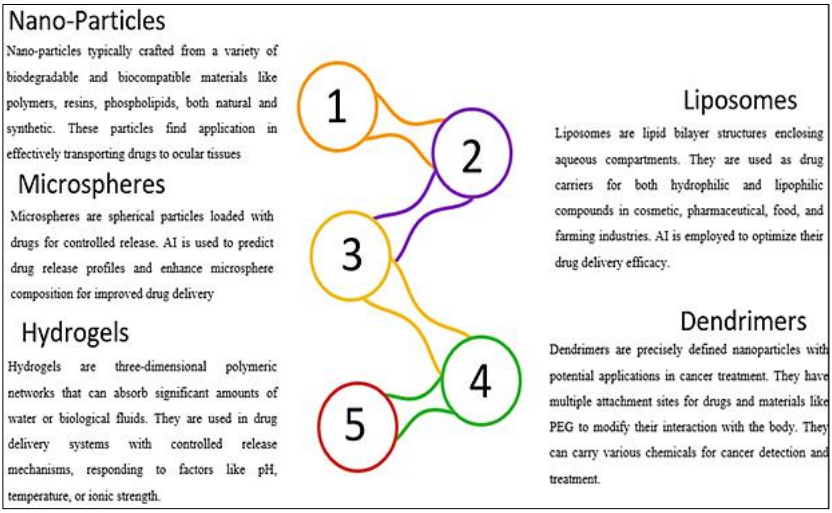
AI-based databases are invaluable for scaling up nanocarriers using automated systems. AI also assists in optimizing nanocarriers and conducting compatibility tests for drug delivery systems, improving drug loading, formulation stability, and retention.(Alshawwa *et al.*, 2022)

Furthermore, AI is applied to predict nanocrystals' properties and behaviours, reducing the need for repetitive experiments through computational techniques like Monte Carlo simulations and molecular dynamics. Such methods enable quantitative measurements in critical experiments.

AI also contributes to the creation of databases for Nano carriers, aiding in the investigation of the relationship between Nano carrier structure and various data types, including toxicity, physical properties, and biological responses. (Patra *et al.*, 2018)

In another study, AI was employed to analyse vascular permeability in nanoparticle-based drug delivery systems, potentially informing the design of more effective systems. Finally, AI, combined with PBPK modelling, can enhance the study of cancer medicine and improve our understanding of the challenges associated with low nanoparticle tumour delivery efficacy. (Kitsios *et al.*, 2023)

Overall, AI has significantly contributed to improving various drug delivery systems like nanoparticles, liposomes, microspheres, dendrimers, and hydrogels.



S. No.	Types of Nano particles	Description
1.	Carbon Nanotubes	Carbon nanotubes are cylindrical structures composed of carbon atoms. They have the potential to be filled and sealed, effectively creating tiny test tubes or possible vehicles for drug delivery.
2.	Nanowires	Glowing silica nanowires are wound around single strands of human hair, appearing fragile. They are about five times smaller than viruses. Nanowires have applications in early detection of breast and ovarian cancers.
3.	Nano cantilevers	In the Nano world, cantilevers are beams anchored at one end, often resembling the surface of a fly's eye. They function as highly sensitive sensors for detecting extremely small molecules in biological fluids.
4.	Nano Shells	Nano shells are hollow silica spheres coated with gold. Scientists can attach antibodies to them, enabling the shells to target specific cells like cancer cells. There's potential for these shells to be filled with drug-containing polymers.
5.	Quantum Dots	Quantum dots are minuscule semiconductor particles that can act as markers for specific cells or molecules in the body. They emit varying wavelengths of radiation based on the type of cadmium used in their cores.
6.	Nano pores	Nano pores have applications in cancer research and treatment. When engineered into particles, these incredibly tiny holes allow DNA molecules to pass through them one strand at a time, enabling precise DNA sequencing and controlled drug release.
7.	Niosome	Noisome are vesicular structures composed of non-ionic surfactants, similar to liposomes but without phospholipids. They are being studied as alternative carriers for various solute particles.

8.	Nano Emulsions	Nano emulsions are colloidal particles with sizes ranging from 10 to 1,000 nm. They serve as drug carriers with stable, lipophilic surfaces. Magnetic nanoparticles can enhance their site-specific targeting, making them valuable in treating infections, liver conditions, cancer, and vaccination.

AI in Nano-robots

Nanorobots are incredibly small synthetic machines capable of sensing, manoeuvring, and performing various tasks on a nanoscale with precise and continuous control. This novel approach to drug delivery differs significantly from traditional methods, offering improvements in targeting, performance, detection, control, and the ability to execute multiple tasks simultaneously. Due to significant advancements in nanotechnology, there has been a growing interest in developing nanorobots equipped with power sources, sensors, and artificial intelligence (AI). (G. Giri *et al.*, 2021)

Nanorobots have displayed great promise in tasks such as detecting toxic substances and applications in therapy and diagnosis. AI plays a crucial role in governing the actions and movements of these nanorobots. To achieve effective control over nanorobot movement, the use of efficient swarm intelligence algorithms is essential. Swarm Intelligence, a subset of Artificial Intelligence, explores algorithms inspired by the behaviour of insects, animals, and birds, enabling collaborative actions without the need for centralized control. Swarm Intelligence methods are specifically tailored for the field of nanorobot artificial intelligence. (Kong *et al.*, 2023)

The remarkable progress in nanotechnology has sparked significant interest in the development of Nano robots, which are equipped with internal or external power sources, sensors, and AI systems. These Nano robots have opened up the possibility of creating implantable robots capable of performing various tasks, including precise drug or gene delivery through blood vessels. The rule structure governing bio-Nano robots includes rules for navigation, collision avoidance, target identification, detection and attachment, drug delivery, mission completion, and activation of the flush-out mode for their removal from the body. However, several challenges remain, such as controlling interactions in complex biological environments and ensuring biocompatibility. (Aggarwal *et al.*, 2022)

Nano robots are primarily composed of integrated circuits, sensors, power supplies, and secure data storage, all managed through computational technologies like AI. These Nano robots are engineered to prevent collisions, identify targets, attach to them, and then exit the body. Advancements in Nano/micro robotics enable them to navigate to specific locations based on

physiological conditions like pH, increasing their effectiveness while reducing systemic side effects. Developing implantable Nano robots for controlled drug and gene delivery involves considerations such as dose adjustment, sustained and controlled release, which can be automated through AI tools like neural networks, fuzzy logic, and integrators. Microchip implants serve both for detecting the implant's location in the body and for programmed drug release. (Soto *et al.*, 2020)

AI in tablets

Artificial intelligence (AI) can play a crucial role in finding the best formulation and understanding the key attributes involved in tablet production. AI is also expected to assist in automating tasks through advanced algorithms and technologies. However, implementing AI presents challenges for regulatory authorities, necessitating a re-evaluation of policies related to good manufacturing practices (cGMP). (Vora *et al.*, 2023c)

Various AI technologies, such as artificial neural networks (ANNs), fuzzy logic, neural networks, and genetic algorithms, are applied in the development of solid dosage forms to improve the understanding of the relationships between input parameters and outcomes in processing and operations. ANN, for instance, is employed for more accurate predictions in solid dosage form development, while genetic algorithms help anticipate results based on input parameters. (Jiang *et al.*, 2022a). AI can also be applied in systemic drug delivery to forecast drug release patterns and explore the impact of critical manufacturing parameters, ensuring consistent quality control measures. (Sohail Arshad *et al.*, 2021)

Integrating AI into drug formulation aids in predicting drug release, reducing the need for numerous test batches, thus saving time and costs in pilot and production processes. AI predicts drug release and dissolution profiles, helping select the best batch for scaling up. Some researchers have used AI algorithms like artificial neural networks (ANNs), support vector machines (SVM), and regression analysis to predict dissolution profiles using data obtained through process analytical technology (PAT) and material attributes, with particle size distribution being a critical factor. ANN is utilized to identify the most accurate models in the evaluation process.

AI in capsules

There's limited literature discussing the use of AI techniques in the development of capsule-based medications. To achieve different drug release profiles, various capsule types, such as hard gelatin soft gelatin, modified release, and enteric capsules, are utilized to encase drug powders. Zhou and

colleagues successfully demonstrated the capability of identifying capsule imperfections using an improved convolutional neural network (CNN) in their study. (Nagy *et al.*, 2023) They manually prepared capsules with various defects like holes, concave heads, uncut bodies, oil stains, shrivelling, locking, or nesting. The enhanced CNN employed L2 regularization and an Adam optimizer to combat model overfitting. Additionally, K-Nearest Neighbour (KNN) and Support Vector Machines (SVM) were used for comparison. The results, as shown in the confusion matrix, revealed an accuracy of up to 97.56% in detecting capsule defects when employing this enhanced CNN model. (Hassanzadeh *et al.*, 2019)

AI in transdermal, parenteral, and mucosal drug delivery

AI can play a crucial role in the development and manufacturing of complex pharmaceuticals like injectables and biologics. It can predict and optimize various physicochemical parameters of drug formulations, such as pH, solubility, stability, and viscosity, by analysing the components, excipients, and manufacturing processes involved. This helps create stable parenteral formulations and improve production quality, efficiency, and consistency. AI can also analyse large datasets from analytical tests to detect and address quality issues early in the manufacturing process.(Jiang *et al.*, 2022b) Additionally, AI can anticipate contamination, stability problems, and regulatory deviations using historical data and real-time process monitoring. It can even optimize equipment maintenance schedules to minimize downtime and enhance output. AI aids in ensuring regulatory compliance by analysing data related to compliance and suggesting process improvements. For example, AI can be used to inspect particles in containers, track their movement, and differentiate between particles and bubbles. Overall, AI enhances pharmaceutical product development, manufacturing, and compliance efforts. (Mohan *et al.*, 2022)

AI in medical devices

Medical devices encompass a variety of tools and machines designed for specific medical purposes. They can be used independently or in conjunction with software and related systems to address patients' medical needs. Artificial Intelligence (AI) has made significant strides in the healthcare sector, transforming it in numerous ways, especially in the context of the COVID-19 pandemic. Personalized medicine and remote health monitoring have gained prominence in many countries, driving the adoption of AI and machine learning in healthcare. Here are some examples of how AI is applied in medical devices: (Waghule *et al.*, 2019)

Diagnostic support: AI algorithms analyse medical imaging data like X-rays, CT scans, and MRIs to assist healthcare professionals in disease detection and diagnosis. For example, AI-powered algorithms can help identify cancerous growths in medical images or spot irregularities in electrocardiograms (ECGs). (Rana *et al.*, 2023)

Remote health monitoring: AI-enabled medical devices remotely track patients' health conditions, providing continuous monitoring of vital signs and other relevant parameters. This is particularly valuable for patients with chronic illnesses, who can receive personalized care from their homes. AI algorithms analyse the collected data and offer insights or alerts to healthcare providers. (Shaik *et al.*, 2023)

Wearable devices: AI is integrated into wearable gadgets like smartwatches, fitness trackers, and biosensors. These devices monitor various health indicators, such as heart rate, sleep patterns, physical activity, and even blood glucose levels. AI algorithms interpret this data, providing users with actionable insights for enhancing their health. (Malche *et al.*, 2022)

Prosthetics and Rehabilitation: AI is utilized in advanced prosthetic devices to offer more natural movement and functionality. Machine learning algorithms can adapt the prosthetic based on user movements. Additionally, AI aids in rehabilitation by analysing motion and providing feedback to patients to enhance their movements and track progress. (Verma *et al.*, 2022)

Surgical support: AI finds applications in surgical devices, assisting surgeons during procedures. Robotic surgical systems, for instance, use AI algorithms to aid surgeons in performing precise and minimally invasive surgeries. AI can also analyse preoperative and intraoperative data to provide real-time guidance and enhance surgical outcomes. (Bodenstedt *et al.*, 2020)

Medication management: AI-powered devices help patients manage their medications effectively. Smart pill dispensers remind patients to take their medications as prescribed, dispense the correct dosages, and monitor adherence. AI algorithms also analyse patient data, including medical history and medication usage, to provide personalized recommendations for medication management.

To address issues after cochlear implant surgery, researchers have considered coating the implants to deliver drugs for inflammation or infections. They used an artificial neural network (ANN) model to predict the parameters and drug release pattern of dexamethasone from these coated implants. The ANN model effectively determined the ideal formulation parameters, speeding up the design process and accurately modelling drug release, closely matching experimental results. (Manda *et al.*, 2019)

Future prospects and applications

The integration of Artificial Intelligence (AI) in drug delivery systems has transformed the pharmaceutical and healthcare landscape, offering innovative solutions to longstanding challenges. As AI technologies continue to advance, they open up new possibilities and future trends that promise to enhance the precision, efficiency, and patient-centricity of drug delivery systems. (S. Patel *et al.*, 2022) This exploration delves into the exciting future trends and applications of AI in drug delivery, envisioning a healthcare ecosystem driven by data-driven decision-making, advanced robotics, nanomedicine, regulatory adaptations, and more. (Davenport *et al.*, 2019)

AI-Driven nanomedicine for precision drug targeting

Nanotechnology has already played a pivotal role in drug delivery by enabling targeted and controlled release of therapeutic agents. AI is poised to take this a step further by optimizing nanomedicine design and deployment. The convergence of AI and nanomedicine will result in highly sophisticated, AI-driven nanoparticles capable of precise drug targeting at the cellular and even subcellular levels. AI algorithms will analyse patient data, including genomics and proteomics, to tailor nanoparticles with optimal drug payloads and surface modifications for specific disease signatures. (Bohr *et al.*, 2020)These smart nanocarriers will navigate through the body, delivering drugs precisely where needed, reducing off-target effects, and enhancing therapeutic outcomes. AI-driven nanomedicine will be particularly valuable in oncology, neurology, and autoimmune diseases where precise drug delivery is critical. It can improve the efficacy of chemotherapy, deliver drugs across the blood-brain barrier, and modulate the immune system with unprecedented precision.

AI-Augmented robotic drug delivery

Robotics in drug delivery systems have demonstrated potential in automating medication dispensing, ensuring precise dosages, and enhancing patient adherence. AI will further augment the capabilities of drug delivery robots. AI-driven robotic drug delivery systems will become highly adaptive and intelligent. (Malik *et al.*, 2023) These robots will not only dispense medications but also assess patient conditions, interpret real-time data from sensors and wearables, and adjust drug regimens accordingly. They will operate autonomously, collaborating with healthcare providers to optimize treatment plans, monitor patient responses, and provide timely interventions in emergencies. AI-augmented robotic drug delivery will find applications in home healthcare, hospitals, and long-term care facilities. It will benefit

patients with chronic conditions, elderly populations, and those requiring complex medication regimens.

AI-Powered patient-centric healthcare

AI will facilitate a paradigm shift towards truly personalized medicine. Patient data, including genetics, medical history, lifestyle, and real-time health metrics, will be continuously analysed by AI systems. (Babel *et al.*, 2021a) These systems will provide real-time treatment recommendations, monitor for adverse events, and adjust therapies based on patient responses. Patients will actively participate in their healthcare decisions, informed by AI-generated insights and predictions. AI-powered patient-centric healthcare will span various therapeutic areas, such as diabetes management, cardiovascular disease, mental health, and rare diseases. It will empower patients to take charge of their health while ensuring they receive the most effective and personalized treatments.

Emergence of AI-driven regulatory frameworks

Regulatory agencies play a critical role in ensuring the safety and efficacy of drugs. The rapid integration of AI in drug delivery necessitates adaptive regulatory frameworks. Regulatory agencies worldwide are evolving to accommodate AI-driven drug delivery systems. They will establish guidelines and validation protocols specific to AI algorithms and their applications in healthcare. (Sauerbrei *et al.*, 2023) AI systems will undergo rigorous validation processes to ensure their reliability and safety in clinical settings. These regulatory frameworks will prioritize transparency, algorithm explain ability, and ethical considerations. The emergence of AI-driven regulatory frameworks will foster trust in AI-powered drug delivery systems, accelerate approvals, and streamline compliance processes. It will pave the way for a smoother integration of AI innovations into healthcare.

AI in drug repurposing for rapid therapeutic discoveries

AI is poised to play a pivotal role in this domain. AI-powered drug repurposing will harness the vast repositories of biomedical data to predict novel therapeutic applications for existing drugs. (Thakkar *et al.*, 2023) Machine learning models will analyse diverse datasets, including genomics, clinical trial data, and drug interactions. AI algorithms will identify hidden connections between drugs and diseases, leading to rapid therapeutic discoveries and reducing the time and cost of drug development. AI-driven drug repurposing will expedite the identification of treatments for rare diseases, explore new indications for off-patent drugs, and enable rapid responses to emerging healthcare crises, such as pandemics or newly discovered diseases. (Zhou *et al.*, 2020)

Ethical considerations and challenges

The future trends and applications of AI in drug delivery systems hold immense promise, but they also raise ethical considerations and potential challenges. (Liu *et al.*, 2022) These include:

1) **Data privacy and security:** The extensive use of patient data in AI-driven drug delivery systems raises concerns about data privacy and security. Ensuring robust data protection measures and obtaining informed consent are essential.
2) **Algorithm bias:** AI algorithms may inadvertently perpetuate biases present in the data they are trained on, potentially leading to healthcare disparities. Addressing bias and ensuring algorithm fairness will be crucial.
3) **Transparency and accountability:** As AI systems become more complex, ensuring transparency and accountability in their decision-making processes is vital. Patients and healthcare professionals should be able to understand and challenge AI-driven recommendations.
4) **Regulatory harmonization:** Achieving global harmonization of AI regulatory frameworks poses a challenge, as different regions may have varying approaches to AI validation and approval.
5) **Education and training:** The healthcare workforce will require education and training to effectively interact with AI systems and leverage their capabilities for improved patient care.

The future trends and applications of AI in drug delivery systems represent a transformative shift towards precision medicine, patient-centric care, and accelerated drug development. (Naik *et al.*, 2022b) AI-driven nano-medicine, robotic drug delivery, patient-centric healthcare, adaptive regulatory frameworks, and drug repurposing are poised to revolutionize the pharmaceutical and healthcare industries. (Johnson *et al.*, 2021) However, addressing ethical considerations and overcoming potential challenges are crucial steps in realizing the full potential of AI in drug delivery systems. By embracing these innovations while maintaining ethical standards, the healthcare ecosystem can unlock the benefits of AI for improved patient care and drug development.

Steps to be taken

Based on the comprehensive exploration of future trends and applications of AI in drug delivery systems, several recommendations can be made to guide the integration of AI into pharmaceutical and healthcare practices:

1) **Invest in AI research and development:** Pharmaceutical companies, research institutions, and governments should prioritize investment in AI research and development. This includes fostering collaborations between AI experts, healthcare professionals, and pharmaceutical researchers to advance AI technologies tailored to drug delivery systems.
2) **Data sharing and collaboration:** Encourage data sharing and collaboration among stakeholders in the pharmaceutical and healthcare industries. Access to diverse and comprehensive datasets is crucial for training AI algorithms effectively. Initiatives that promote data sharing while respecting patient privacy should be supported.
3) **Ethical guidelines and training:** Develop and adhere to ethical guidelines for the use of AI in drug delivery systems. Additionally, healthcare professionals should receive training to effectively interact with AI systems and understand their decision-making processes.
4) **Regulatory adaptations:** Regulatory agencies should work collaboratively to establish adaptive regulatory frameworks specific to AI-driven drug delivery systems. These frameworks should prioritize transparency, algorithm explain ability, and ethical considerations.
5) **Patient education and engagement:** Empower patients to be active participants in their healthcare decisions. AI-driven personalized medicine requires patient engagement and informed consent. Develop patient education materials to explain the role of AI in their treatment.

Conclusion

In conclusion, the future of AI in drug delivery systems holds tremendous promise, ushering in a new era of precision, efficiency, and patient-centric care. The envisioned future trends and applications of AI, from AI-driven nanomedicine for precise targeting to AI-augmented robotic drug delivery, signify a transformative shift in healthcare and pharmaceuticals. These innovations have the potential to not only streamline drug development processes but also empower patients to actively participate in their treatment decisions, ultimately leading to improved therapeutic outcomes. Moreover, the emergence of AI-driven regulatory frameworks is essential to ensure the safety and ethical use of these technologies in healthcare. These frameworks

will provide the necessary guidelines for validation, transparency, and ethical considerations, fostering trust among stakeholders and facilitating the integration of AI innovations. However, it is vital to acknowledge the ethical considerations and potential challenges accompanying this AI-driven future. Data privacy, algorithm bias, transparency, regulatory harmonization, and workforce education must all be carefully addressed to maximize the benefits of AI while upholding ethical standards. The future trends and applications of AI in drug delivery systems offer a compelling vision of a healthcare landscape that is personalized, efficient, and ethically responsible. By embracing these innovations and addressing the accompanying ethical considerations, the pharmaceutical and healthcare industries can harness the transformative power of AI to enhance patient care, accelerate drug development, and shape a more promising future for healthcare worldwide.

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Chapter - 4
A Comprehensive Review on Artificial Intelligence in Pharmaceutical Industry

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Chapter - 4

A Comprehensive Review on Artificial Intelligence in Pharmaceutical Industry

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Abstract

Artificial Intelligence (AI) focuses in producing intelligent modelling, which helps in imagining knowledge, cracking problems and decision making. Recently, AI plays an important role in various fields of pharmacy like drug discovery, drug delivery formulation development, polypharmacology, hospital pharmacy, etc. In drug discovery and drug delivery formulation development, various Artificial Neural Networks (ANNs) like Deep Neural Networks (DNNs) or Recurrent Neural Networks (RNNs) are being employed. Several implementations of drug discovery have currently been analysed and supported the power of the technology in quantitative structure-property relationship (QSPR) or quantitative structure-activity relationship (QSAR). In addition, de novo design promotes the invention of significantly newer drug molecules with regard to desired/optimal qualities. In the current review article, the uses of AI in pharmacy, especially in drug discovery, drug delivery formulation development, polypharmacology and hospital pharmacy are discussed.

Keywords: Artificial intelligence, artificial neural network, drug discovery, drug delivery research

Introduction

Artificial Intelligence (AI) is a stream of science related to intelligent machine learning, mainly intelligent computer programs, which provides results in the similar way to human attention process [1]. This process generally comprises obtaining data, developing efficient systems for the uses of obtained data, illustrating definite or approximate conclusions and self-corrections/adjustments [2]. In general, AI is used for analyzing the machine learning to imitate the cognitive tasks of individuals. AI technology is exercised to perform more accurate analyses as well as to attain useful interpretation [3]. In this perspective, various useful statistical models as well

as computational intelligence are combined in the AI technology [4]. The progress and innovation of AI applications are often associated to the fear of unemployment threat. However, almost all advancements in the applications of AI technology are being celebrated on account of the confidence, which enormously contributes its efficacy to the industry. Recently, AI technology becomes a very fundamental part of industry for the useful applications in many technical and research fields. The emergent initiative of accepting the applications of AI technology in pharmacy including drug discovery, drug delivery formulation development and other healthcare applications have already been shifted from hype to hope [5-6]. The uses of AI models also make possible to predict the *in vivo* responses, pharmacokinetic parameters of the therapeutics, suitable dosing, etc. [7]. According to the importance of pharmacokinetic prediction of drugs, the uses of in silico models facilitate their effectiveness and inexpensiveness in the drug research [8]. There are two key classes of AI technology developments [9]. The first one comprises the conventional computing methodologies including expert systems, which are capable of simulating the human experiences and illustrating the conclusions. from the principles, like expert systems [10]. The second one comprises the systems, which can model the mode of brain functioning employing the artificial neural networks (ANNs). In specific, various ANNs like deep neural networks (DNNs) or recurrent neural networks (RNNs) control the evolutions of AI technology. In Merck Kaggle [11] and NIH Tox21 challenge [12], DNN issues show the greater predictivity than the baseline machine learning methodologies [13]. The machine learning employs suitable statistical methodologies with the capability to learn with or devoid of being unequivocally programmed [14]. In addition, de novo design promotes the invention of newer drug molecules with regard to optimal or desired qualities. In the current review article, the uses of AI in pharmacy, especially in drug discovery, drug delivery formulation development, polypharmacology and hospital pharmacy are discussed.

Classification of AI

AI can be classified into two different ways: according to calibre and their presence [15-16]. According to their ability, AI can be categorized as:

i) **Artificial Narrow Intelligence (ANI) or Weak AI:** It performs a narrow range task, i.e., facial identification, steering a car, practicing chess, traffic signalling, etc.

ii) **Artificial General Intelligence (AGI) or Strong AI:** It performs all the things as humans and also known as human level AI. It can simplify human intellectual abilities and able to do unfamiliar task.

iii) **Artificial Super Intelligence (ASI):** It is smarter than humans and has much more activity than humans drawing, mathematics, space, etc.

According to their presence and not yet present, AI can be classified as follows:

i) **Type 1:** It is used for narrow purpose applications, which cannot use past experiences as it has no memory system. It is known as reactive machine. There are some examples of this memory, such as a IBM chess program, which can recognize the checkers on the chess playing board and capable of making predictions.

ii) **Type 2:** It has limited memory system, which can apply the previous experiences for solving different problems. In automatic vehicles, this system is capable of making decisions there are some recorded observations, which are used to record further actions, but these records are not stored permanently.

iii) **Type 3:** It is based upon "Theory of Mind". It means that the decisions that human beings make are impinged by their individual thinking, intentions and desires. This system is non-existing AI.

iv) **Type 4:** It has self-awareness, i.e., the sense of self and consciousness. This system is also non-existing AI.

Neural networks and ANNS

The learning algorithm of neural networks (from input data) takes two different forms mainly. The classes of neural networks are as follows [17].

i) **Unsupervised learning:** Here the neural network is submitted with input data having recognised pattern. It is used for organizational purpose. The unsupervised learning algorithm uses 'Self Organizing Map' or 'Kohonen'. This is known as very useful modeling for the searching of relationships amongst the complex data sets.

ii) **Supervised learning:** This kind of neural network is illustrated with the sequences of harmonizing inputs and outputs.

It is used for learning relationship connection between the inputs and the outputs. It shows its usefulness in formulation to measure the cause and effects linking between input-output. It is the most frequently employed ANNs and is entirely linked with the back propagation learning rule. This learning algorithm is known as the outstanding methodology for the prediction as well as classification jobs. A simple mathematical processing unit called neuron is the main part of the neural network. Every input

possesses an associated weight having relative importance and calculates the weighted sum of all the inputs as output. This output is then forwarded to another neuron after being modified by a transformation function. The whole processing is called a perceptron (a feed-forward system). A neural network having many neurons is organized into network architectures. The most famous and prosperous network is multilayer perceptron network. In this network system, the identical neurons are arranged in such a way that in one layer, the outputs are presented and in the subsequent layer, the inputs are presented. There are one or more secret layers, which can be introduced between the input and output layers. In theory, amount of secret layers can be attached according to solitary need. In practice, multiple layers are needed in case of applications with extensive nonlinear behaviour.

ANN is one of the computational modelling figured from hundreds of single units of artificial neurones associated with the constituents comprising the neural structure, which are known as processing elements as they participate in information processing [18]. ANN methodology presents a potential modeling procedure, in particular for the data sets of non-linear links commonly encountered in the pharmaceutical research [19-21]. For the model specification analyses, ANNs don't necessitate acquaintance of the data source. However, they frequently have many weightages that should be analyzed. They also necessitate larger training sets. Additionally, ANNs can mix as well as add in both the literature and the investigational data to resolve the problems. Recently, ANN models are being hybridized with other kinds of simpler models [22]. For example, a recently proposed combination of neural networks and logistic regression allow the generation of hybrid linear/nonlinear classification surfaces and the identification of possible strong interactions that may exist between the attributes (also known as covariates in the Logistic Regression literature which define the classification problem. All these hybrid models perform reasonable well for a given set of databases. The prospective uses of ANNs in pharmacy are wide ranged from the data analyses via the modeling of pharmaceutical quality control [23]. ANNs are also proved functional for the uses in the drug designing, especially in molecular modeling and QSAR [24]. It is also used in formulation optimization processes for dosage form designing and in biopharmaceutical analyses, such as pharmacokinetic modeling, pharmacodynamic modeling, *in vitro-in vivo* correlation analysis, etc. [25]

Fuzzy Logic and Neurofuzzy Logic

According to the conventional logic, proposal may be true or false. The hypothesis behind the logic lies either in or totally outside the "true" set.

When the hypothesis lies within the “true” set, the membership function is denoted as “1” and when the hypothesis lies outside the “true” set, the membership function is denoted as “0”. The basic concept of fuzzy logic is promoted by Lotfi Zadeh in the 1960s [26]. In contrast to the conventional logic, the fuzzy logic is not limited to be 0 or 1. However, any continuous value in-between these limits can be taken here. When 20 °C temperature is taken as “comfortable”, according to the conventional logic temperature of 19 or 21 °C, which remain outside this set, are “uncomfortable”. But, according to fuzzy logic, 17 °C may obtain a membership of 0.4 in the “hot” set as well as 0.6 in the “cold” set. This logic is very useful in process control. For the automated circulations by the arteries and venous, the automated system based on fuzzy logic for drug releasing has been framed and analysed [27]. Fuzzy hemodynamic management modules have already been employed for the assessment of the condition of patients to report the regulation of the arterial as well as pulmonary pressures. This can be used to monitor the cardiac output of patients. The fuzzy logic-based automated system offers a comparative faster reaction and more effectual haemodynamic control. In addition, the uses of supervisory-fuzzy rule-dependent adaptive control system is considered as a potential way for controlling the multiple drug hemodynamic process [28]. When the fuzzy logic system is strongly combined with a neural network, it is called as neurofuzzy logic system. Here, the capability of neural networks of learning from data and the ability of fuzzy logic of expressing complex concepts intuitively are combined properly. It has data mining capability. The neurofuzzy logic also presents neural network having 2 extra layers for the fuzzification of inputs as well as defuzzification of outputs. In a research, the simulation of probucol absorption via the lipid formulations has been studied by means of neurofuzzy networkings [29]. According to the outcome of the research, the probucol releasing rate from the lipid formulations was found to be significantly lesser in comparison with that of the self-emulsifying formulations. The adaptive neurofuzzy network model together with *in vitro-in vivo* correlation tool demonstrated the competent predictive presentation and the prospective for the development of complex relationships as well as interpolates the pharmacokinetic constraints.

Principal Component Analysis (PCA)

PCA is another AI based model for decreasing the dataset-dimensionality by preserving as much ‘variability’ (i.e., statistical information) as possible and at the same time, PCA modelling minimizes the loss of information. PCA modelling translates into searching newer

variables, which are linear functions of those in the original dataset by generating newer uncorrelated variables so that maximize the variance, successively. Searching of such newer variables, the principal components reduce the resolving of an eigenvector or eigenvalue problem [30-31]. PCA can be based on either the covariance matrix or the correlation matrix and the main applications of PCA are descriptive in nature, rather than the inferential uses. Recent years, PCA is well-known for using as a 'hypothesis generating' AI tool generating a useful statistical mechanics frame for modelling of biological systems without the requirement for strong a priori theoretical assumptions, which makes PCA of paramount significance for drug discovery research by a systemic perspective overcoming too narrow reductionist approaches [32].

Advantages of AI technology

The potential advantages of AI technology are as follows [33]:

i) **Error minimization:** AI assists to decrease the errors and increase the accuracy with more precision. Intelligent robots are made of resistant metal bodies and capable of tolerating the aggressive atmospheric space, therefore, they are sent to explore space.

ii) **Difficult exploration:** AI exhibits its usefulness in the mining sector. It is also used in the fuel exploration sector. AI systems are capable of investigating the ocean by defeating the errors caused by humans.

iii) **Daily application:** AI is very useful for our daily acts and deeds. For examples, GPS system is broadly used in long drives. Installation of AI in Androids helps to predict what an individual is going to type. It also helps in correction of spelling mistakes.

iv) **Digital assistants:** Now-a-days, the advanced organizations are using AI systems like 'avatar' (models of digital assistants) for the reduction of human needs. The 'avatar' can follow the right logical decisions as these are totally emotionless. Human emotions and moods disturb the efficiency of judgement and this problem can be overcome by the uses of machine intelligence.

v) **Repetitive tasks:** In general, human beings can perform single task at a time. In contrast to the human beings, machines are capable of performing multi-tasking jobs and can analyze more rapidly in comparison to the human beings. Various machine parameters, i.e., speed and time can be adjusted according to their requirements.

vi) **Medical uses:** In general, the physicians can assess the condition of patients and analyze the adverse effects and other health risks associated with the medication with the help of AI program. Trainee surgeons can gather knowledge by the applications of AI programs like various artificial surgery simulators (for examples, gastrointestinal simulation, heart simulation, brain simulation, etc.

vii) **No breaks:** Unlike human beings who have the capacity of working for 8 h/day with breaks, the machines are programmed in such a way that these are capable of performing the work in a continuous manner for long hours devoid of any kinds of confusions and boredom.

viii) **Increase technological growth rate:** AI technology is widely used in most of the advanced technological innovations worldwide. It is capable of producing different computational modelling programs and aims for the invention of the newer molecules. AI technology is also being used in the development of drug delivery formulations.

ix) **No risk:** In case of working at the risky zone like fire stations, there are huge chances of causing harm to the personnel engaged. For the machine learning programs, if some mishap happens then broken parts can be repairable.

x) **Acts as aids:** AI technology has played a different function by serving children as well as elders on a 24x7 basis. It can perform as teaching and learning sources for all.

xi) **Limitless functions:** Machines are not restricted to any boundaries. The emotionless machines can do everything more efficiently and, also produce more accurately than the human beings.

Conclusion

During past few years, a considerable amount of increasing interest towards the uses of AI technology has been identified for analyzing as well as interpreting some important fields of pharmacy like drug discovery, dosage form designing, polypharmacology, hospital pharmacy, etc., as the AI technological approaches believe like human beings imagining knowledge, cracking problems and decision making. The uses of automated workflows and databases for the effective analyses employing AI approaches have been proved useful. As a result of the uses of AI approaches, the designing of the new hypotheses, strategies, prediction and analyses of various associated factors can easily be done with the facility of less time consumption and inexpensiveness.

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Chapter - 5
A Pharmacological Review of Psychotic Disorder Medications: Antipsychotics and Mood Stabilizers

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Chapter - 5

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Abstract

This comprehensive review explores the pharmacological interventions for psychotic disorders, focusing on antipsychotics and mood stabilizers. Antipsychotics, including typical and atypical agents, are pivotal in managing symptoms such as hallucinations, delusions, and agitation in psychotic patients. They exert their effects primarily through dopamine receptor blockade, mitigating psychotic symptomatology while also exhibiting varying degrees of adrenergic, cholinergic, and histaminergic interactions. Mood stabilizers, exemplified by lithium, sodium valproate, and carbamazepine, play crucial roles in bipolar disorder management, offering acute and prophylactic treatment options. Understanding their mechanisms, adverse effects, and interactions is vital for optimizing therapeutic outcomes in psychiatric practice.

Keywords: Antipsychotics, antimanics, hallucinations, mood stabilizers, lithium, bipolar, delusions

Introduction

Psychosis and mania are two distinct yet interconnected mental health conditions that can significantly impact an individual's thoughts, emotions, and behavior. While psychosis involves a loss of touch with reality, characterized by hallucinations, delusions, and disorganized thinking, mania is marked by an elevated or irritable mood, increased energy levels, and impulsive behavior.

Understanding Psychosis:

Psychosis is a mental state characterized by a profound disruption in a person's thoughts and perceptions, leading to a detachment from reality. Symptoms of psychosis may include hallucinations, which are sensory experiences such as hearing voices or seeing things that are not present, and

delusions, which are fixed false beliefs that are resistant to reason or contrary evidence. Additionally, individuals experiencing psychosis may exhibit disorganized thinking and speech, making it difficult for them to communicate coherently or engage in logical reasoning.

Psychosis can occur as a primary symptom of psychiatric disorders such as schizophrenia, schizoaffective disorder, and bipolar disorder with psychotic features. It can also manifest secondary to medical conditions, substance abuse, or extreme stress. Early recognition and intervention are crucial in managing psychosis, as untreated psychosis can lead to functional impairment, social withdrawal, and increased risk of self-harm or harm to others.

Mania

Mania is a mood disorder characterized by an abnormally elevated or irritable mood, accompanied by increased energy levels and a heightened sense of self-importance. Individuals experiencing mania may engage in risky or impulsive behaviors, such as overspending, reckless driving, or substance abuse. They may also exhibit rapid speech, racing thoughts, and a decreased need for sleep.

Mania is a defining feature of bipolar disorder, specifically bipolar I disorder, where episodes of mania alternate with episodes of depression. However, mania can also occur in other psychiatric conditions such as bipolar II disorder, schizoaffective disorder, and substance-induced mood disorders. The management of mania often involves a combination of pharmacotherapy, psychotherapy, and lifestyle interventions aimed at stabilizing mood, reducing symptoms, and preventing relapse.

Psychosis and mania represent profound disruptions in mental health, impacting individuals' perceptions and behaviors. While psychosis entails a detachment from reality through hallucinations, delusions, and disorganized thinking, mania is characterized by heightened mood, energy, and impulsivity. These conditions often co-occur within disorders like schizophrenia and bipolar disorder, necessitating comprehensive understanding and treatment approaches to address their multifaceted nature. Effective management involves early recognition, intervention, and a holistic approach encompassing pharmacotherapy, psychotherapy, and social support.

I. Anti psychotics

These are the drugs that are used to treat psychotic disorders including schizophrenia (neuroleptic, ataractic, major tranquilizer).

- **Synonyms:** Major tranquilizers, Neuroleptics.

Classification [1]: There are various anti-psychotic drugs as shown in figure 01.

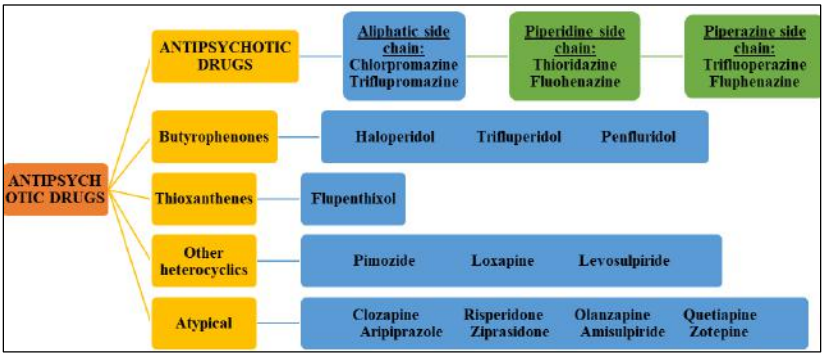

Fig 1: Classification of Antipsychotics

Pharmacological actions [2]

1. CNS effects

A. In nonpsychotic

Chlorpromazine (CPZ)

- Indifference to surroundings.
- Paucity of thought.
- Psychomotor slowing.
- Emotional quietening.
- Reduction in initiative.
- Tendency to go off to sleep from which the subject is easily arousable.
- Spontaneous movements-minimized but slurring of speech, ataxia or motor incoordination does not occur = Neuroleptic syndrome.
- The typical antipsychotics have potent D2 receptor blocking property and producing extrapyramidal motor side effects = Neuroleptic drugs.

B. In psychotics

CPZ

- Reduces irrational behaviour.
- Agitation.
- Aggressiveness.

- Controls psychotic symptomology.
- Disturbed thought and behaviour-gradually normalized.
- Anxiety-relieved.
- Hyperactivity, hallucinations and delusions-suppressed.

▪ All phenothiazines, thioxanthenes and butyrophenones have **same antipsychotic efficacy** but **potency differs** in equieffective doses.

▪ Aliphatic and piperidine side chain phenothiazines have 1.low potency 2. More sedation.

The sedative effect is produced faster than antipsychotic effect. Tolerance develops to sedative effect but not to antipsychotic effect.

- Performance and intelligence-unaffected.
- Vigilance-impaired.
- No consistent in sleep architecture.
- Disturbed sleep-normalised.
- Lowers seizure threshold.
- Precipitate fits in untreated epileptics.
- Temperature control is knocked off making patient poikilothermic.
- The medullary respiratory and vital centres are not affected except at higher doses.
- Phenothiazines and butyrophenones except thioridazine have potent antiemetic action.

Mechanism of action: Following figure: 02 shows mechanism of anti-psychotics.

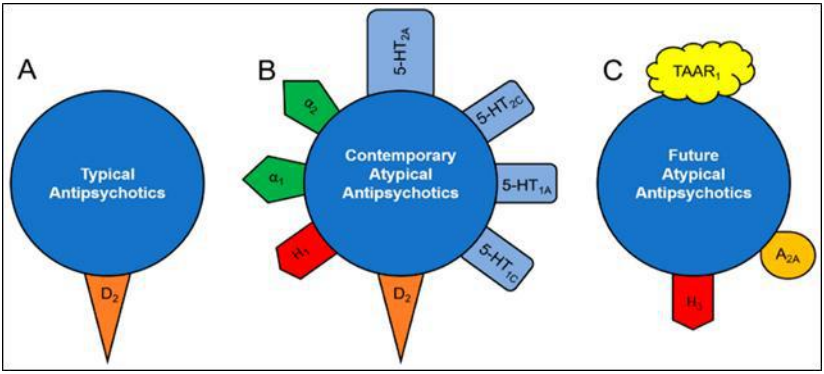

Fig 2: Mechanism of action of Psychotics

2. ANS effects

- Neuroleptics have varying degree of alpha-adrenergic blocking activity.

CPZ = triflupromazine = thioridazine > clozapine > fluphenazine > haloperidol > trifluoperazine > pimozide, i.e., more potent compounds have lesser α blocking activity.

Thioridazine > CPZ > triflupromazine > trifluoperazine = haloperidol.

- **Anticholinergic property** of neuroleptics is **weak.**
- The phenothiazines have **weak H1-antihistaminic** and **anti-5-HT actions.**

3. Local anaesthetics

- **CPZ-potent local anaesthetic** as procaine, but not used because of it's irritant action.

4. CVS Effects

- Neuroleptics produce **postural hypotension** by both central and peripheral action on sympathetic tone (more marked by parenteral administration) which is not prominent in psychotics.
- Partial tolerance to hypotension occurs on prolonged use. Reflex tachycardia accompanies hypotension. High doses of CPZ shows following effects as shown in figure: 03.

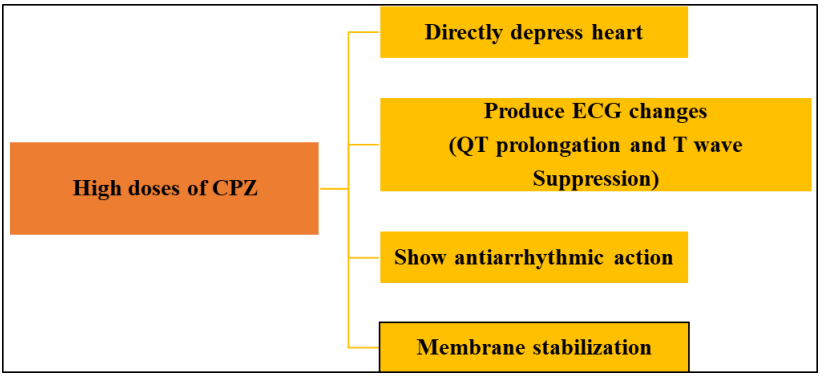

Fig 3: Effects of high dose of chlorpromazine

5. Endocrine effects [4]

- Neuroleptics consistently increase prolactin release by blocking the inhibitory action od DA on pituitary lactotrophs.

Result: Galactorrhoea, gynaecomastia.

CPZ

- GH, gonadotropins and ADH are reduced.
- ACTH response to stress is subdued.

Antipsychotic drugs do not affect blood sugar in general but CPZ and few others can cause.

- Impairment of glucose tolerance.
- Aggravate diabetes.
- Elevate serum triglycerides.
- Weight gain & Accentuation of insulin resistance.

Tolerance and Dependence

- Tolerance to the sedative and hypotensive actions develops within days or weeks, but maintenance doses for therapeutic effect in most psychotics remain fairly unchanged over years, despite increased DA turnover in the brain.
- Neuroleptics are hedonically (pertaining to pleasure) bland drugs, lack reinforcing effect so that chronic recipients do not exhibit drug seeking behaviour.
- Physical dependence is probably absent, though some manifestations on discontinuation have been considered withdrawal phenomena.

Pharmacokinetics [3]

Absorption: Oral absorption is unpredictable. Good absorption on IM and IV administration. Bioavailability is low.

Protein binding: Plasma and tissue protein binding are well.

Volume of distribution: Large (20 L/kg)

Metabolism: In liver mainly by CYP2D6 enzyme.

Duration of action: 6-8 hours.

Half-life: 18-30 hours.

The duration of clinical benefit is much longer relative to the elimination half-life, because of tight binding to D2 receptors.

Excretion: Urine and bile.

Neuroleptics [4]: The effects and doses of some neuroleptics are shown in table: 01.

- Neuroleptic drugs, also known as antipsychotics, are medications primarily used to manage symptoms of psychiatric disorders such as schizophrenia, bipolar disorder, and severe depression.
- They work by altering the balance of certain neurotransmitters in the brain, particularly dopamine and serotonin, to alleviate symptoms like hallucinations, delusions, and disorganized thinking. Neuroleptics are classified into two main groups: typical (first-generation) and atypical (second-generation) antipsychotics, each with varying side effect profiles and mechanisms of action.
- While effective in controlling symptoms, neuroleptic drugs can also cause significant side effects such as weight gain, sedation, movement disorders, and metabolic changes.
- Close monitoring by healthcare professionals is essential to manage these adverse effects and optimize treatment outcomes.
- Despite their efficacy, the use of neuroleptics requires careful consideration of individual patient factors and potential risks, particularly in vulnerable populations such as the elderly and children.
- Additionally, ongoing research aims to develop safer and more targeted antipsychotic medications with fewer side effects and improved efficacy.
- Proper education and collaboration between patients, caregivers, and healthcare providers are crucial for ensuring the safe and effective use of neuroleptic drugs in managing psychiatric disorders.

Table 1: Pharmacology of each Neuroleptic drug

Drug name	Potency	Dose and dosage form	Actions	Side effects	Class of drug
1. Triflupromazine	More potent than CPZ	10mg tab 10mg/ml inj	Antiemetic	Acute muscle dystonia in children	Aliphatic side chain phenothiazine
2. Thioridazine	low	25,50,100mg tab.	Central anticholinergic action	Incidence of extrapyramidal side effects, cardiac arrhythmias, interference with male sexual function, eye damage	Phenothiazine
3. Trifluoperazine, fluphenazine	high	1,5,10mg tab. 1mg tab., 2.5mg/5ml elixir.	Minimum autonomic actions	Extrapyramidal and tardive dyskinesia	Piperazine side chain phenothiazine
4. Haloperidol	high	1.5,5,10mg tab., 5mg/mlinj., 2,5,10mg tab2mg/ml oral liq., 10mg/ml drops.	Few autonomic effects **Uses:** acute schizophrenia, Huntington's disease, Gilles de la Tourette's syndrome	Anticholinergic effects and sedation, weight gain, amenorrhea.	Typical antipsychotic
5. Trifluperidol	More potent than haloperidol	0.5mg tab., 2.5mg/ml inj.	Anticonvulsant and antiemetic action.	Anorexia, cholestatic jaundice, blurred vision, anxiety.	Powerful neuroleptic
6. Penfluridol	Moderate	20mg tab	Chronic schizophrenia	Affective withdrawal and social maladjustment	Long-acting neuroleptic
7. Flupentixol	Less sedating than CPZ	0.5,1,3mg tab., 20mg/ml inj.	Schizophrenia, other psychoses, particularly in withdrawn and apathetic patients	Constipation, xerostomia, fatigue, tremor	Neuroleptic

Atypical antipsychotics [5]: Kinetics, effects and doses of atypical antipsychotics are shown in table: 02.

Table 2: Pharmacology of Atypical drugs

Drug	Action	Kinetics	Indications	Side effects	Dose
1. Clozapine	Blocks D4, 5-HT2, α adrenergic receptors, moderately potent anticholinergic, Significant H1 blocker	By CYP1A2, CYP2C19, CYP3A4 Half-life: 12hrs	Schizophrenia, reduces the risk of recurrent suicidal behaviour.	Higher incidence of agranulocytosis (0.8%), other blood dyscrasias: Metabolic complication like weight gain, Hyperlipidaemia, precipitates diabetes, High dose-Seizures, sedation, unstable BP, tachycardia, urinary incontinence, few cases of myocarditis	25-100mg/day, max 300mg/day
2. Risperidone	D2 + 5-HT2 receptor blockade, high affinity for α1, α2 and H1 receptors	Oral bioavailability of tablet is 94%, 77% plasma bound	Schizophrenia, bipolar I acute manic or mixed episodes as monotherapy	Postural hypotension, BP rise, prolactin levels rise disproportionately during risperidone therapy, agitation, weight gain, incidence of new-onset, increased risk of stroke in the elderly.	Dose changes from 0.25mg to 2mg per day based on indications for different age groups.
3. Olanzapine	Blocks D2, 5-HT2, α1, α2, Muscarinic, H1 receptors.	By CYP1A2 and glucuronyl transferase Half-life: 24-30hrs	Mania	Dry mouth, constipation, few extrapyramidal side effects, little rise in prolactin levels, more epileptogenic, weight gain, higher risk of impairing glucose tolerance or worsening diabetes, elevating serum triglyceride,	Dose changes from 5mg to 20mg per day based on the indication and age.

				incidence of stroke in elderly	
4. Quetiapine	blocks 5-HT1A, 5-HT2, D2, α1, α2, H1 receptors in the brain, but D2 blocking is low:	Mainly by CYP3A4.	Acute mania as well as in bipolar depression.	Postural hypotension, urinary retention/incontinence in few, weight gain, rise in blood sugar are moderate, QTc prolongation, risking arrhythmia only at high dose	Min dose: 50mg per day Max dose: 800mg per day.
5. Aripiprazole	partial agonist at D2 and 5-HT1A receptor, but antagonist at 5-HT2 receptor.	By CYP3A4 as well as CYP2D6; dose needs to be halved in patients receiving ketoconazole or quinidine, and doubled in those taking carbamazepine Half-life:~ 3 days	Schizophrenia as well as mania and bipolar illness	Insomnia, little tendency to weight gain, rise in blood sugar, moderate prolongation of Q-Tc interval occurs at higher doses, nausea, dyspepsia, constipation, light-headedness	Min dose: 2mg per day Max dose: 30mg per day.
6. Ziprasidone	D2 + 5-HT2A/2C + H1 + α1 blocking activity. Antagonistic action at 5-HT1D + agonistic activity at 5-HT1A receptors along with moderately potent inhibition of 5-HT and NA reuptake indicates some anxiolytic and antidepressant property as well	Half-life: ~8 hours	Schizophrenia, mania	Mildly sedating, causes modest hypotension, little weight gain or blood sugar elevation, nausea, vomiting, prolongation of Q-T interval occurs imparting potential to induce serious cardiac arrhythmias.	Min dose: 20mg per day Max dose: 80mg per day.

7. Amisulpiride	high affinity for D2 (and D3) receptors and has low-affinity for 5-HT2 receptors.	Absorption: oral. Excretion: unchanged in urine. Half-life: 12 hours.	Antidepressant	Insomnia, anxiety and agitation, risk of weight gain and metabolic complications are lower, Q-T prolongation.	50-300 mg/day in 2 doses for schizophrenia with predominant negative symptoms. For acute psychosis 200-400 mg BD.

Adverse effects [5]

1. **CNS**
   - Drowsiness, lethargy, mental confusion.
   - Tolerance to sedative effect.
   - Increased appetite and weight gain.
   - Aggravation of seizures in epileptics, non-epileptics also develop seizures at higher doses.
2. **CVS**
   - Postural hypotension, palpitations, inhibition of ejaculation.
   - Q-T prolongation, cardiac arrhythmias at higher doses.
3. **Anticholinergic**
   - Dry mouth, blurring of vision.
   - Constipation, urinary hesitancy in elderly male.
4. **Endocrine**
   - Hyperprolactinemia.
   - Lower Gn levels.
   - Amenorrhoea, infertility, galactorrhoea and gynaecomastia occur infrequently after prolonged treatment.
5. **Metabolic effects**
   - Elevation of blood sugar and triglyceride levels as a consequence of chronic therapy with certain antipsychotics.
   - High risk of precipitating diabetes or worsening it.
   - Raised triglyceride level is another consequence of insulin resistance.
   - Cardiovascular mortality among schizophrenics is higher.
6. **Extrapyramidal disturbances**
   - **Parkinsonism:** With typical manifestations-rigidity, tremor, hypokinesia, mask like facies, shuffling gait.
   - **Acute muscular dystonia:** Bizarre muscle spasms, mostly involving linguo-facial muscles grimacing, tongue thrusting, torticollis, locked jaw.
   - **Akathisia:** Restlessness, feeling of discomfort, apparent agitation manifested as a compelling desire to move about, but without anxiety is seen.

- **Malignant neuroleptic syndrome:** It occurs rarely with high doses of potent agents. The patient develops marked rigidity, immobility, tremor, hyperthermia, semiconsciousness, fluctuating BP and heart rate; myoglobin may be present in blood.
- **Tardive dyskinesia:** It occurs late in therapy, sometimes even after withdrawal of the neuroleptic: manifests as purposeless involuntary facial and limb movements like constant chewing, pouting, puffing of cheeks, lip licking, choreoathetoid movement, manifestation of progressive neuronal degeneration along with super sensitivity to DA.

7. Miscellaneous

- Weight gain often occurs due to long-term antipsychotic therapy
- Sugar and lipids may tend to rise.
- Blue pigmentation of exposed skin, corneal and lenticular opacities, retinal degeneration (more with thioridazine) occur rarely after long-term use of high doses of phenothiazines.

Interactions [5]

1) Neuroleptics potentiate *all CNS depressants-hypnotics, anxiolytics, alcohol, opioids and anti-histaminics.* **Overdose symptoms** may occur.
2) Neuroleptics **block the actions of levodopa** and **direct DA agonists** in *parkinsonism.*
3) **Antihypertensive action** of clonidine and methyldopa is reduced, probably due to central α2 adrenergic blockade.
4) Phenothiazines and others are **poor enzyme inducers**-no significant pharmacokinetic interactions occur. Enzyme inducers *(barbiturates, anticonvulsants)* can **reduce blood levels** of neuroleptics.

II. Antimanic

These are the drugs used to treat mania and to break into cyclic affective disorders. The figure: 06 shown below shows different anti-manic drugs [6].

Classification [6]

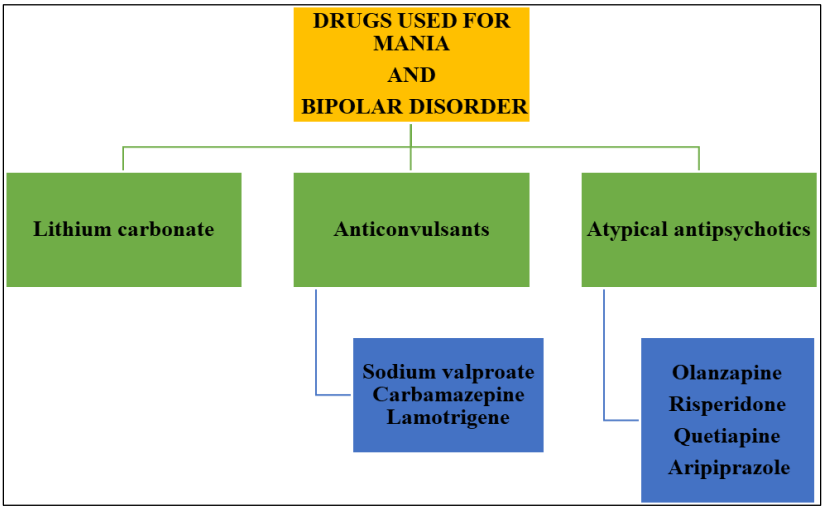

Fig 6: Classification of drugs used for Mania & Bipolar Disease

Actions and Mechanism [7]

1. CNS Effects

- Lithium has practically **no acute effects in normal individuals** as well as in **bipolar patients.**
- It is **neither sedative nor euphoriant**; but on prolonged administration, it acts as a mood stabiliser in bipolar disorder.

Indications: In acute mania, continued treatment prevents cyclic mood changes. The reduced sleep time of manic patients is normalized.

Mechanism: The mechanism of antimanic and mood stabilizing action of lithium is not known. The proposed mechanism was Li+ partly replaces body Na+ and is nearly equally distributed inside and outside the cells (contrast Na+ and K+ which are unequally distributed); this may affect ionic fluxes across brain cells or modify the property of cellular membranes. However, relative to Na+ and K+ concentration, the concentration of Li+ associated with therapeutic effect is very low.

b) Lithium decreases the presynaptic release of NA and DA in the brain of treated animals without affecting 5-HT release. This may correct any imbalance in the turnover of brain monoamines.

The fallowing figure:05 shows the mechanism of Lithium on CNS.

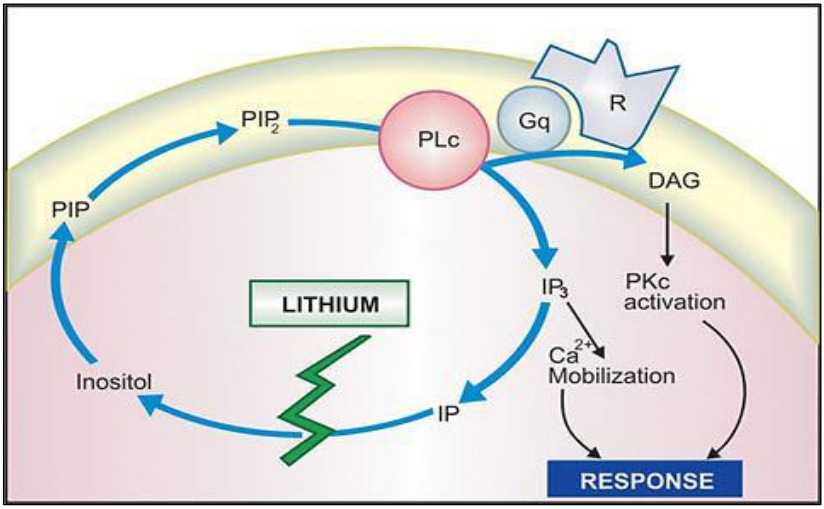

Fig 5: Mechanism of Lithium PIP-Phosphatidyl inositol phosphate; PIP2-Phosphatidyl inositol bisphosphate; IP3-Inositol trisphosphate; IP-Inositol-1-phosphate; PLc-Phospholipase C; DAG-Diacylglycerol; PKc-Protein Kinase C; Gq-Coupling Gq protein; R-Neurotransmitter receptor.

2. Other actions

- Lithium inhibits the action of ADH on distal tubules in the kidney and causes a diabetes insipidus.
- An insulin-like action on glucose metabolism is exerted.
- Leukocyte count is increased by lithium therapy.
- Lithium inhibits release of thyroid hormones resulting in feedback stimulation of thyroid through pituitary.
- Majority of Li+ treated patients remain in a state of compensated euthyroidism, but few get decompensated and become clinically hypothyroid.

Pharmacokinetics [8]

Absorption: Slowly but well absorbed orally.

- No plasma protein binding and metabolism

Distribution: In extracellular water, gradually enters cells penetrates into brain, ultimately uniform distribution in total body water is attained.

- CSF concentration of Li+ = ½ of plasma concentration.

Apparent volume of distribution: At steady-state averages 0.8 L/kg.

Excretion: Kidney in much. Nearly 80% of the filtered Li+ is reabsorbed in the proximal convoluted tubule. Lithium is excreted in sweat and saliva as well, and secreted in breast milk.

Mothers on lithium should not breastfeed

- After a single dose of Li+, its urinary excretion is rapid for 10–12 hours, followed by a much slower phase lasting several days (t½ =16–30 hours).

Renal clearance of lithium =1/5 of creatinine clearance

- On repeated medication, steady-state plasma concentration is achieved in 5-7 days.
- Levels are higher in older patients and in those with renal insufficiency.

Note: There is marked individual variation in the rate of lithium excretion. Thus, with the same daily dose, different individuals attain widely different plasma concentrations. However, in any individual the clearance remains fairly constant over time. Since the margin of safety is narrow, monitoring of serum lithium concentration is essential for optimising therapy. Serum lithium level is measured 12 hours after the last dose to reflect the steady-state concentration.

Dose

0.5-0.8 mEq/L is considered optimum for maintenance therapy in bipolar disorder.

0.8-1.1 mEq/L is required for episodes of acute mania.

Toxicity symptoms occur frequently when serum levels exceed 1.5 mEq/L.

Adverse effects [8]

Side effects are common, but are mostly tolerable. Toxicity occurs at levels only marginally higher than therapeutic levels.

1. Nausea, vomiting and mild diarrhoea occur initially, can be minimized by starting at lower doses.
2. Thirst and polyuria are experienced by most, some fluid retention may occur initially, but clears later.
3. Fine tremors are noted even at therapeutic concentrations.

4. CNS toxicity manifests as plasma concentration rises producing coarse tremors, giddiness, ataxia, motor incoordination, nystagmus, mental confusion, slurred speech, hyper-reflexia.

Overdose symptoms are regularly seen at plasma concentration above 2 mEq/L. In acute intoxication these symptoms progress to muscle twitching, drowsiness, delirium, coma and convulsions. Vomiting, severe diarrhoea, albuminuria, hypotension and cardiac arrhythmias are the other features.

Treatment: It is symptomatic. There is no specific antidote. Osmotic diuretics and sod. bicarbonate infusion promote Li+ excretion. Haemodialysis is indicated if serum levels are > 4 mEq/L.

5. On long-term use, some patients develop renal diabetes insipidus. Most patients gain some body weight.

Goitre has been reported in about 4%. This is due to interference with release of thyroid hormone → fall in circulating T3, T4 levels → TSH secretion from pituitary → enlargement and stimulation of thyroid. Enough hormone is usually produced due to feedback stimulation so that patients remain euthyroid. However, few become hypothyroid. Lithium induced goitre and hypothyroidism does not warrant discontinuation of therapy; can be easily managed by thyroid hormone supplementation.

6. Lithium is contraindicated during pregnancy: foetal goiter and other congenital abnormalities, especially cardiac, can occur; the newborn is often hypotonic.
7. At therapeutic levels, Li+ can cause reduction of T-wave amplitude. At higher levels, SA node and A-V conduction may be depressed, but arrhythmias are infrequent. Lithium is contraindicated in sick sinus syndrome.
8. Lithium can cause dermatitis and worsen acne.

Interactions [9]

1) Diuretics (thiazide, furosemide) by causing Na+ loss promote proximal tubular reabsorption of Na+ as well as Li+ → plasma levels of lithium rise. Potassium sparing diuretics cause milder Li+ retention.
2) Tetracyclines, NSAIDs and ACE inhibitors can also cause lithium retention.
3) Lithium reduces pressor response to NA.
4) Lithium tends to enhance insulin/sulfonylurea

5) induced hypoglycaemia.
6) Neuroleptics, including haloperidol, have been frequently used along with lithium without problem. However, sometimes, the combination of haloperidol and lithium produces marked tremor and rigidity. The neuroleptic action appears to be potentiated by lithium.

Uses [9]

1) **Acute mania:** Though lithium is effective in controlling acute mania, response is slow and control of plasma levels is difficult during the acute phase. Maintenance lithium therapy is generally given for 6-12 months to prevent recurrences.
2) **Prophylaxis in bipolar disorder:** Lithium has proven efficacy in bipolar disorder: is gradually introduced and maintained at plasma concentration between 0.5-0.8 mEq/L. Such treatment lengthens the interval between cycles of mood swings: episodes of mania as well as depression are attenuated, if not totally prevented in 70% patients.
3) Lithium is used in many other recurrent neuropsychiatric illnesses, cluster headache and as adjuvant to antidepressants in resistant non bipolar major depression.

Alternatives to lithium [10]

1) Sodium valproate A reduction in manic relapses is noted when valproate is used in bipolar disorder.

- It is now a first line treatment of acute mania in which high dose valproate acts faster than lithium and is an alternative to antipsychotic ± benzodiazepine.
- It can be useful in those not responding to lithium or not tolerating it.
- Patients with rapid cycling pattern may particularly benefit from valproate therapy.
- Valproate has a favourable tolerability profile, and now its use as prophylactic in bipolar disorder has exceeded that of lithium.
- Combination of valproate with an atypical antipsychotic has high efficacy in acute mania.
- Divalproex, a compound of valproate, is more commonly used due to better gastric tolerance. Dosage guidelines are the same as for epilepsy.

2) **Carbamazepine:** Soon after its introduction as antiepileptic, carbamazepine (CBZ) was found to prolong remission in bipolar disorder.

- Its efficacy in mania and bipolar disorder has now been confirmed.
- Carbamazepine is less effective than lithium or valproate in acute mania.
- Initiation of therapy with high doses needed for efficacy produce neurotoxicity and are poorly tolerated.
- Compared to lithium and valproate, efficacy of carbamazepine for long-term prophylaxis of bipolar disorder and suicides is less well established.
- Nevertheless, it is a valuable alternative/adjunct to lithium. The dose and effective plasma concentration range is the same as for treatment of epilepsy.

3) **Lamotrigine:** There is now strong evidence of efficacy of this newer anticonvulsant for prophylaxis of depression in bipolar disorder.

- Lamotrigine is not effective for treatment as well as prevention of mania.
- Extensively used in the maintenance therapy of type II bipolar disorder, because in this condition risk of inducing mania is minimal.
- Lamotrigine can be combined with lithium to improve its efficacy. The tolerability profile of lamotrigine is favourable.

4) **Atypical antipsychotics:** Olanzapine, risperidone, aripiprazole, quetiapine, with or without a BZD, are now the first line drugs for control of acute mania, except cases requiring urgent parenteral therapy, for which the older neuroleptics are still the most effective.

Aripiprazole has recently emerged as the favoured drug for treatment of mania in bipolar I disorder, both as monotherapy as well as adjuvant to lithium or valproate. Maintenance therapy with aripiprazole prevents mania, but not depressive episodes. Lack of metabolic effects, favours its long-term use.

Olanzapine is also approved for maintenance therapy of bipolar disorder. Though both manic and depressive phases are suppressed, it is not considered suitable for long-term therapy due to higher risk of weight gain, hyperglycaemia, etc.

Strong evidence of efficacy of **Quetiapine** has emerged in bipolar depression. Combination of an atypical antipsychotic with valproate or lithium has demonstrated high efficacy in acute phases as well as for maintenance therapy of bipolar disorder.

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Chapter - 6
A Review on Pharmacokinetics: Understanding Drug Movement in the Body

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Chapter - 6

A Review on Pharmacokinetics: Understanding Drug Movement in the Body

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Abstract

Pharmacokinetics, the study of drug movement in the body, encompasses absorption, distribution, metabolism, and excretion (ADME). This process is crucial in determining a drug's efficacy and safety profile. Absorption involves the transfer of a drug from its delivery site to the bloodstream, influenced by various physiological factors and routes of administration. Distribution depends on factors like solubility, protein binding, and tissue permeability, with drugs reaching an equilibrium between plasma and tissue fluids. Metabolism, primarily occurring in the liver, involves biotransformation to inactive or active metabolites, impacting a drug's duration and effectiveness. Excretion, predominantly through urine and bile, eliminates drugs and their by products from the body. Understanding pharmacokinetics aids in optimizing drug dosing and therapeutic outcomes while minimizing adverse effects.

Keywords: Pharmacokinetics, absorption, distribution, metabolism, excretion, biotransformation, elimination

Pharmacokinetics

Introduction

The term pharmacokinetics comes from the Greek terms pharmaco, which means drug, and kinesis, which means movement in relation to the drug. Pharmacokinetics studies how drugs are absorbed, distributed, and eliminated. Pharmacokinetics is schematically represented in figure-01.

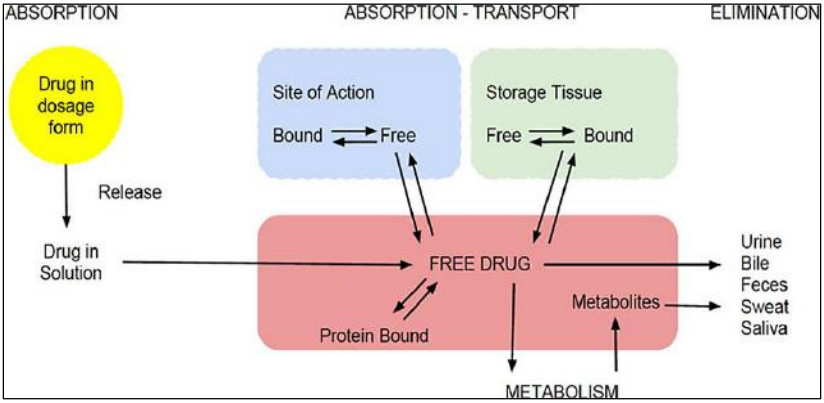

Fig 1: Schematic representation of Pharmacokinetics

- The medicine is absorbed (A), distributed (D), and removed from the body through from the body through metabolism and excretion.
- The pharmacokinetics of a drug describes its absorption, distribution, metabolism, and excretion within the body.
- Understanding these processes is essential for determining the drug's concentration at the site of action and its duration of action.
- Factors such as route of administration, formulation, patient characteristics, and drug interactions influence the pharmacokinetic profile of a drug.
- Pharmacokinetic parameters, including bioavailability, volume of distribution, clearance, and half-life, are quantitatively assessed to optimize dosing regimens and ensure therapeutic efficacy while minimizing adverse effects.

Pharmacokinetic parameters

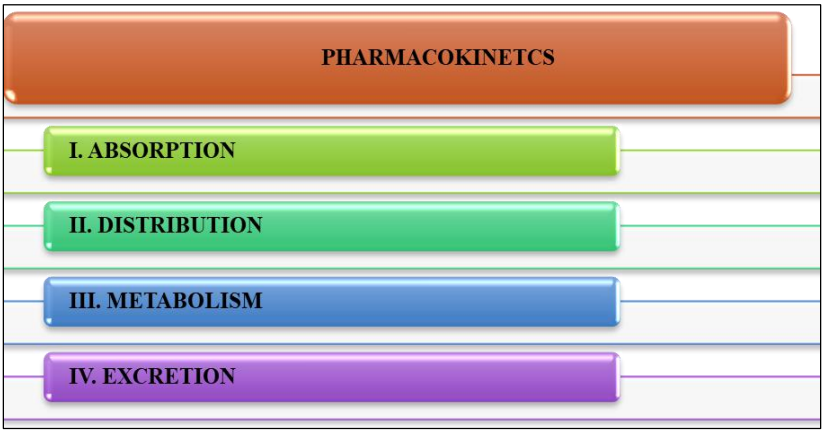

Fig 2: Pharmacokinetic parameters

I. Absorption

- Absorption refers to the transfer of a medicine from its delivery site to the bloodstream. Not only is it a fraction of the prescribed dose received.
- The rate of absorption is essential. Drugs must pass biological membranes to be absorbed, unless administered intravenously.

Physiological factors impacting absorption include

1) Routes of Drug Administration.
2) Membrane Physiology.

I. Characteristics of cell membrane

II. Transporters

3) Surface area.
4) Gastric emptying time.
5) Gastrointestinal motility.
6) Splanchnic blood flow.
7) Drug Stability in the GIT.
8) Impact of food and nutrition.
9) Disease status.
10) Drug Interaction.

1. Routes of administration

Oral administration: The drug is taken orally.

Features: Absorption occurs along the entire length of GIT (i.e., huge surface area).

- **Absorption site:** Gastrointestinal epithelium.

Buccal/Sublingual: The drug is administered orally or sublingually.

- **Advantages:** Quick absorption without first-pass impact.

This route is used to administer drugs that are very lipid-soluble and have a high first-pass impact when swallowed (for example; nitroglycerine).

- **Absorption site:** buccal/sublingual mucosa.

Inhalation: Inhalation involves inhaling the medication and absorbing it through the lungs.

- Features include broad surface area and quick absorption.
  - Avoids first-pass metabolism.
  - e.g.: Volatile and gaseous medications are administered this way.
- **Absorption site:** Alveoli in the lung.

Intranasal: The medicine is administered into the nasal cavity.

- **Absorption site:** Nasal membrane.

Rectal: Drugs are delivered into the rectum, such as suppositories.

  - The absorption pathway avoids first-pass metabolism.
  - Rectal wall epithelia serve as absorption sites.

Parenteral routes: Biotechnology-derived medications (e.g., insulin, erythropoietin, somatotropin) are administered parenterally since they are too labile in the GI tract to be given orally.

Intravenous (IV): Administering a medication straight into the bloodstream.

- Benefits include no absorption, 100% bioavailability, and quick commencement of action.

Intramuscular (IM): Medication injected into skeletal muscle; absorption is quicker than SC but slower than IV.

- Absorption site: striated muscular fibers.

Subcutaneous (SC): Drug absorption from subcutaneous tissues.

- **Absorption site:** Subcutaneous tissue.

2. Membrane physiology

- Medicine molecules must pass through cell membranes to be absorbed systemically. The permeability of a medication depends on its molecular structure and properties.
- Cell membranes are composed of phospholipids, carbohydrates, and protein groups.
- P-glycoprotein, a transporter, protects the body from harmful substances by removing drugs from the intestines, maintaining blood brain barrier integrity, and removing drugs from the kidneys and liver.

3. Surface area

Larger surface area facilitates faster absorption, pH range of 5-8 ideal for protein digestion, making it impossible to orally take protein-based drugs like insulin. Absorbent surface area influences absorption.

4. Gastric emptying time

- The time taken for stomach contents to enter the duodenum is influenced by gastric motility and pyloric sphincter activity.
- Acidic drugs absorb faster, while basic drugs absorb easily.
- Acidic drugs require more gastric emptying time, while basic drugs require less.

5. Gastrointestinal motility

The GIT plays a crucial role in the absorption and bioavailability of medications through the alimentary canal, preventing excessive mobility and facilitating small intestine carrier mediated transport mechanisms.

6. Splanchnic blood flow

Food increases splanchnic blood flow, causing higher plasma concentrations of certain medicines like propranolol, chloramphenicol, and lithium carbonate, while reducing absorption of ampicillin, aspirin, and L-dopa in a hypovalent state.

7. Drug stability in the GIT

Enzyme metabolism or degradation, as well as chemical hydrolysis, can impair medication absorption. Gastric acid can destroy certain antibiotics, such as penicillin.

8. Impact of food and nutrition

Water-soluble vitamins, like B-12 and folic acid, are more efficiently absorbed in the stomach when combined with intrinsic factors, while grape juice's flavonoids, like naringin, inhibit cytochrome P-450 enzymes.

9. Disease state

- Patients on tricyclic antidepressants and antipsychotic medicines have decreased GI motility, which delays drug absorption.
- In achlorhydric patients, weak-base medications stay undissolved in the stomach due to a lack of sufficient acid, for example; Dapsone

10. Drug interactions

- Anticholinergic drugs (propantheline bromide) may delay stomach emptying and motility of the small intestine.
- Tricyclic antidepressants and phenothiazines with anticholinergic side effects reduce peristalsis and stomach emptying, which may delay medication absorption.
- Antacids should not be taken with cimetidine or tetracycline because they impair absorption.

Bioavailability

Bioavailability is the rate and extent of drug absorption from a dose form, measured by the concentration-time curve. Blood or its excretion in urine the fraction (F) of a medication supplied that remains unaltered in the systemic circulation. Drugs administered intravenously have 100% bioavailability, while oral consumption may result in partial absorption.

a) The absorbed drug may be incompletely absorbed.

b) The absorbed drug may go via first pass metabolism in the intestinal wall/liver or be eliminated in bile.

Bioequvivalence

Bioequivalence refers to the difference in bioavailability between oral drug formulations. Two drug preparations are considered bioequivalent if the rate and extent of bioavailability are not significantly different under proper test conditions. Bioavailability is influenced by factors such as solubility, particle size, crystal structure, and physical features. Low solubility drugs have lower bioavailability. Microfine tablets absorb aspirin more quickly due to smaller particle size. Bioavailability variation is crucial for medications with low safety margins or precise dosage management.

II. Distribution

Once a medicine enters the bloodstream, it is transported to previously untreated tissues via a concentration gradient from plasma to tissues.

A drug's distribution depends on its

- Solubility of lipids.
- Ionization at physiological pH (pKa).

Binding to plasma and tissue protein

- Transporter presence.
- Regional blood flow variances.
- The drug moves until it reaches an equilibrium between unbound drug in plasma and tissue fluids. Elimination causes a corresponding reduction in both.

Apparent distribution volume (V) Assuming the body is a single, homogeneous compartment of volume V, the medicine will be distributed uniformly.

$$V = \frac{\text{□□□□ □□□□□□□□□□□□ □.□}}{\text{□□□□□□ □□□□□□□□□□□□□□□}}$$

Redistribution

- Lipid-soluble medications are primarily delivered to organs with high blood flow, such as the brain, heart, and kidneys.
- The medication is absorbed by less vascular but bulkier tissues (e.g., muscle and fat), resulting in lower plasma concentrations and withdrawal from highly perfused areas.
- Redistribution of a medication to a highly perfused organ can end its activity.
- Drugs with higher lipid solubility undergo quicker redistribution.

Factors affecting the volume of drug distribution

- **Lipid:** The drug's water partition coefficient.
- PKa value of the medication.
- Degree of plasma protein binding.
- Preference for various tissues.
- The fat-to-lean body mass ratio can fluctuate. Factors to consider include age, gender, obesity, and diseases such as heart failure, uremia, and cirrhosis.

- Thiopentone sod. administered intravenously has a short lived anesthetic effect due to redistribution within minutes.
- Oral diazepam or nitrazepam provide a transient hypnotic effect lasting 6-8 hours due to redistribution, while having an elimination time of more than 30 hours.
- When a drug is administered repeatedly over time, the low perfusion high-capacity sites gradually fill up, resulting in a longer-acting drug.

Penetration into Brain and CSF

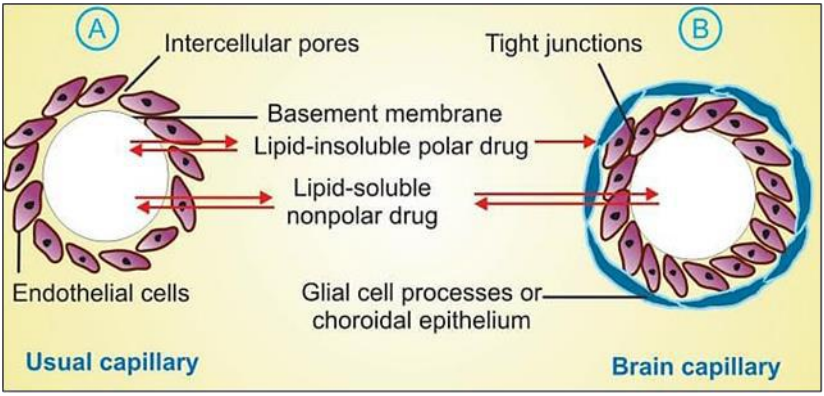

Fig 3: Penetration of drug into usual capillary and Brain capillary

A. big paracellular gaps in capillaries allow for the diffusion of even big lipid-insoluble molecules.

B. Capillary forming the blood-brain or blood-CSF barrier.

- The blood-brain barrier (BBB) in the brain and choroid plexus prevents non-lipid-soluble molecules from passing through.
- Capillary endothelial cells and neural tissue cover these capillaries, forming a blood-CSF barrier.
- Only lipid-soluble medicines can enter the central nervous system. Efflux transporters and enzymes in the brain and choroidal arteries expel harmful xenobiotics.
- The BBB is deficient at certain sites, but drug exit is unrestricted.
- Bulk flow of CSF and drug is facilitated by arachnoid villi and nonspecific organic anion and cation transport processes as shown in fig.03.

Passage across placenta

Placental membranes allow lipophilic medicines to flow freely, while restricting hydrophilic ones. Placental efflux transporters limit foetal exposure. The placenta's role in drug metabolism may reduce exposure. Nonlipid-soluble medications can enter fetuses, and incomplete barriers can influence newborns.

Plasma protein binding

- Drugs have a reversible binding affinity to plasma proteins.
- Acidic medications often bind to plasma albumin, while basic drugs bind to the α1 acid glycoprotein.
- Albumin binding has a higher quantitative significance.
- The extent of binding varies by molecule and cannot be generalized across pharmacological or chemical classes.
- Even minor chemical changes can significantly impact protein binding.
- For example, some benzodiazepines have a binding percentage of:

  Flurazepam (10%) Alprazolam 70 percent.

  Lorazepam 90%. Diazepam 99 percent.
- Increasing medication concentrations can gradually saturate binding sites, resulting in reduced fractional binding.
- Percentage binding refers to the average therapeutic plasma concentrations of a medication.

Plasma protein binding has important clinical implications including

i) Plasma proteins-attached drugs are primarily found in the vascular compartment, distributing in lesser volumes due to their inability to pass membranes.

ii) The bound fraction, while not useful, maintains equilibrium with the free drug in plasma and dissociates when concentration decreases due to elimination, resulting in temporary medication storage.

iii) High protein binding drugs have longer half-life due to active retrieval by liver or kidney tubules, reduced drug concentration in arteries, higher renal clearance, and faster plasma protein binding.

- Examples are penicillin excretion (elimination time of 30 minutes) and lidocaine metabolism.

- Hemodialysis cannot remove highly protein bound medicines; necessitating specialized therapy for poisoning.

iv) Plasma concentrations of drugs, including bound and free drugs, should consider protein binding degree when relating to in vitro active concentrations, such as antimicrobial MIC.

v) Drugs can bind to albumin sites, causing displacement interactions. High-affinity drugs can double free form concentration, but this is temporary and clinical significance is achieved in highly bound drugs with limited distribution volume.

vi) Plasma concentrations of drugs, including bound and free drugs, should be compared to in vitro active medication concentrations like antimicrobial's MIC, considering protein binding degree.

vii) Displacement interactions between medications on albumin molecules can cause temporary doubled free form concentrations, causing drug diffusion, metabolism, or elimination, with most minor and clinically significant for heavily bound medications.

- Aspirin replaces sulfonylureas.
- Indomethacin and phenytoin displace warfarin.
- Sulfonamides and vitamin K displace bilirubin, causing kernicterus in neonates.
- Aspirin replaces methotrexate.

vi) Hypoalbuminemia and uraemia can affect drug binding, with hypoalbuminemia reducing binding and uraemia increasing it in pregnant women and inflammatory patients.

Tissue storage

Tissue storage Drugs may also accumulate in specific organs by active transport or get bound to specific tissue constituents Drugs sequestrated in various tissues are unequally distributed, tend to have larger volume of distribution and longer duration of action. Some may exert local toxicity due to high concentration, e.g., tetracyclines on bone and teeth, chloroquine on retina, streptomycin on vestibular apparatus, emetine on heart and skeletal muscle. Drugs may also selectively bind to specific intracellular organelle, e.g.; tetracycline to mitochondria, chloroquine to nuclei.

III. Metabolism or biotransformation

Definition

Drugs are eliminated from the body either unchanged through the kidneys and bile, or they may undergo chemical changes that allow them to be more easily excreted. The process of undergoing chemical changes is called biotransformation, or metabolism.

The majority of hydrophilic medications, such as pancuronium, neostigmine, streptomycin, etc. are mostly eliminated unaltered and undergo minimal biotransformation.

Sites: The primary site for drug metabolism is Liver Others- Kidney, intestine, lungs, plasma.

Drug biotransformation

Others-Kidney, intestine, lungs, plasma.

The drug which is in lipophilic or hydrophobic form gets converted into hydrophilic metabolites (when entered cells).

Drug biotransformation may result in the following outcomes

i) Deactivation or inactivation

The majority of drugs and their active metabolites, such as ibuprofen, paracetamol, lidocaine, chloramphenicol and its active metabolite 4-hydroxypropranolol, are rendered inactive or less active.

ii) An active drug from active metabolite

Many drugs undergo partial conversion to one or more active metabolites; the effects that are felt are the result of the combined action of the parent drug or medication and its active metabolites.

e.g., Codeine $\longrightarrow$ morphine

iii) Activation of inactive drug

Few drugs are inactive, but must be transformed into active metabolites, known as prodrugs, for stability, improved bioavailability, reduced toxicity, and selective activation at the action site.

Note: Prodrug means an inactive drug precursor that is converted in the body to the active drug form

e.g.; Levodopa (prodrug) $\longrightarrow$ Dopamine

Biotransformation reactions can be classified into:

a) Phase I/Non synthetic/Functionalization reactions:

A metabolite is created or exposed, and it might be active or inert

b) Synthetic/Conjugation/Phase II reactions

The drug conjugates with an endogenous radical, and the metabolite is largely inert, with a few exceptions.

e.g.; the glucuronide conjugate of morphine and the minoxidil sulfate conjugate are both active.

Phase-I

i) **Oxidation:** This is the drug molecule breaking apart by engulfing a water molecule. Certain drugs epoxide, metabolites produced by CYP are detoxified by epoxide hydrolases. The liver, plasma, and other tissues all undergo hydrolysis.

Ex: Choline esters, procaine, lidocaine, procainamide, aspirin, carbamazepine epoxide.

ii) **Reduction:** This reaction, which is the reverse of oxidation, includes the opposite action of cytochrome P-450 enzymes and reduces alcohols, aldehydes, and quinones.

Ex: Drugs like Chloralhydrate, chloramphenicol, halothane, and warfarin are the main drugs that are reduced.

iii) **Hydrolysis:** This involves breaking down a drug molecule by absorbing a water molecule. Similarly, amides and polypeptides are hydrolyzed by amidases and peptidases. Moreover, epoxide hydrolases are present to detoxify epoxide metabolites of some drugs produced by CYP oxygenases. Hydrolysis takes place in the liver, intestines, plasma, and other tissues.

Ex: Choline esters, procaine, lidocaine, procainamide, aspirin, carbamazepine-epoxide, pethidine, and oxytocin.

iv) **Cyclization:** This involves ring structure formation from a straight-chain compound, such as proguanil.

v) **Decyclization:** This involves opening up the ring structure of the cyclic drug mothe medication or its stage I metabolize an endogenous substrate-usually an amino acid or carbohydrate-to create a polar, highly ionized organic acid that is easily eliminated in the bile or urine. Conjugation reactions demand a lot of energy.

Phase-II

i) **Conversion of glucuronides the primary synthetic reaction that a class of UDP-glucuronosyl transferases (UGTs) performs is this one.** Glucuronic acid, which is produced from glucose, can be readily conjugated with compounds that have hydroxyl or carboxylic acid groups. Chloramphenicol, aspirin, paracetamol, diazepam, lorazepam, morphine, and metronidazole are a few examples. This system is used not just by medicines but also by endogenous substrates such as thyroxine, bilirubin, and steroidal hormones. Glucuronidation raises the drug's molecular weight, facilitating its excretion in bile. Bile secreted drug glucuronides that bacteria can hydrolyze the stomach, where the medication is reabsorbed and suffers the same outcome. This enterohepatic cycling of the medication that prolongs its effects, such as oral contraceptives and phenolphthalein.

ii) **Acetylation Acetyl coenzyme-A is used to conjugate compounds containing amino or hydrazine residues**, such as sulfonamides, isoniazid, PAS, dapsone, hydralazine, clonazepam, and procainamide. N-acetyl transferases (NATs) are regulated by several genes, and the rate of acetylation exhibits genetic variability (slow and fast acetylators).

iii) **Methylated Methionine and cysteine acting as methyl donors,** such as adrenaline, histamine, nicotinic acid, methyldopa, captopril, and mercaptopurine, can methylate amines and phenols.

iv) **Conjugation of sulfate Sulfotransferases (SULTs),** such as those found in chloramphenicol, methyl-dopa, adrenal, and sex steroids, sulfate phenolic compounds and steroids.

v) **Conjugation of glycine** Salicylates, nicotinic acid, and other medicines containing carboxylic acid group, however this is not a prominent metabolic pathway.

vi) **Conjugation of glutathione Glutathione-S-transferase (GST)** does this by creating a mercapturate. Usually, it's a small route. Nevertheless, it functions to deactivate extremely reactive quinone or epoxide intermediates produced during the metabolism of specific medications, such as paracetamol. The glutathione supply is depleted when a significant amount of these intermediates are generated (during poisoning or during enzyme stimulation); harmful adducts are then formed with tissue components, resulting in tissue destruction.

vii) **Synthesis of nucleotides and ribonucleosides** this route is crucial for the activation of many antimetabolites of pyrimidines and purines that are utilized in cancer treatment. As shown in Fig. 3.1, the majority of medications are metabolized in a variety of ways, either simultaneously or sequentially. Reaction rates via several channels can differ significantly. A medicine may produce a range of metabolites—some greater, some less. A drug's stereoisomers may metabolize in distinct ways and at different rates, for example. R-warfarin is slowly broken down by sidechain reduction, but S-warfarin experiences fast ring oxidation.

Presystemic first pass metabolism

This is the process by which a medication is metabolized as it moves from the site of absorption into the systemic circulation. Drug metabolizing enzymes are present in the intestinal wall and liver (where they initially enter through the portal vein) of all oral medications. metabolism prior to systemic in the stomach and liver can be prevented by administering the medication parenterally, sublingually, or via transdermal application. Limited presystemic metabolism, however, can happen in the lungs (for drugs that enter the bloodstream by any route) and skin (for drugs that are applied transdermally). The degree of first pass metabolism varies throughout medications.

Drugs with a high first pass metabolism have the following characteristics

i) Oral dosage is significantly higher than sublingual or parenteral dose.

ii) Because of variations in the degree of first pass metabolism, there is a noticeable individual variation in the oral dose.

iii) Oral bioavailability appears to be higher in patients suffering from severe liver disease.

iv) Giving a medication concurrently with another medication that competes with it in first pass metabolism, such as propranolol and chlorpromazine, increases the drug's oral bioavailability.

Table 1: Induction & inhibition of the drug

CYP	Substrates	Inducers	Inhibitors
1A2	Acetaminophen, antipyrine, caffeine	Smoking, charcoal-broiled foods, cruciferous vegetables	Galangin, furafylline, fluvoxamine

2A6	Coumarin, tobacco nitrosamines, nicotine	Rifampin, phenobarbital	Menthofuran, methoxsalen
2B6	Artemisinin, bupropion, cyclophosphamide, ketamine	Phenobarbital, cyclophosphamide	Ticlopidine, clopidogrel
2C8	Taxol, all-trans-retinoic acid	Rifampin, barbiturates	Trimethoprim
2C9	Celecoxib, flurbiprofen, ibuprofen, tolbutamide, sulfaphenazole, S- warfarin	Barbiturates, rifampin	Tienilic acid, sulfaphenazole
2C18	Tolbutamide, phenytoin	Phenobarbital	N-benzylnirvanol,
2C19	Diazepam, propranolol, naproxen, omeprazole	Barbiturates, rifampin	Fluconazole
2D6	Bufuralol, bupranolol, clomipramine, clozapine, codeine, encainide, timolol, tricyclic antidepressants	Unknown	Quinidine, paroxetine
2E1	Acetaminophen, chlorzoxazone, enflurane, halothane, ethanol	Ethanol, Isoniazid	4-Methylpyrazole, disulfiram
3A4	Acetaminophen, amiodarone, cortisol, lidocaine, nifedipine, spironolactone.	Barbiturates, carbamazepine, glucocorticoids, pioglitazone, phenytoin, rifampin	Azamulin, fluconazole, ketoconazole, troleandomycin

Induction and inhibition are two key mechanisms that modulate drug metabolism. Induction refers to the process by which certain drugs or substances stimulate the production of drug-metabolizing enzymes in the liver, leading to an increased rate of metabolism of other drugs. This can result in reduced efficacy or shorter duration of action for affected drugs. In contrast, inhibition involves the suppression of drug-metabolizing enzymes, prolonging the half-life and potentially increasing the toxicity of co-administered drugs. Understanding these phenomena is crucial for predicting and managing drug interactions and optimizing therapeutic outcomes. Few examples are listed in the table-01.

IV. Excretion

The exit of a systemically absorbed medication is known as excretion. Medications and their byproducts are eliminated in:

Pee via the kidney: For the majority of medications, it is the most significant route of excretion (see below).

i) **Urine:** Other than the unabsorbed fraction, the majority of the medication seen in feces comes from bile. OATP is the liver's active transporter of organic acids into bile, particularly medication

glucuronides. MRP2 and OCT transport organic bases, P-gp transports lipophilic medicines, and nonspecific active transport pathways transport steroids. The bile preferentially eliminates bigger molecules (MW > 300). The majority of free drugs in the gut, including those produced by enteric bacteria by glucuronide deconjugation, are reabsorbed via enterohepatic cycle and eventually excreted in urine. Only the remainder is eliminated in the feces. Enterohepatic cycling extends the drug's duration in the body. Drugs with high concentrations in bile include erythromycin, ampicillin, rifampin, tetracycline, oral contraceptives, vecuronium, and phenolphthalein. Certain medications, such as anthracene purgatives and heavy metals, are excreted straight through the colon.

ii) **Exhaled air:** The lungs remove gases and volatile liquids, such as general anaesthetics and alcohol, regardless of their type. tiv lipid solubility. The gas/vapor's alveolar transport is determined by its partial pressure in blood. The lungs also capture and expel particulate debris that enters the bloodstream.

iii) **Saliva and sweat:** These play a small role in drug excretion. These secretions contain considerable levels of lithium, potassium iodide, rifampin, and heavy metals. The majority of the saliva, including the medicine, is swallowed and meets the same fate as an orally administered drug.

iv) **Milk:** Drug excretion in milk is not relevant for the mother, but for the suckling newborn. Drugs with a higher lipid solubility and lower protein binding cross more effectively. Milk has a lower pH (7.0) and higher concentration of basic medicines compared to plasma. Breastfeeding exposes the infant to a limited amount of medications, and most drugs can be administered to nursing women without harming the baby. However, it is recommended to only deliver medications to nursing women when absolutely necessary. The list includes both safe drugs and those that should be avoided while breastfeeding or with additional precautions.

Renal Excretion

- The kidney is responsible for excreting water-soluble compounds. Drug or metabolite levels in urine are determined by glomerular filtration, tubular reabsorption, and tubular secretion Net renal (glomerular filtration + tubular = excretion secretion)-tubular reabsorption.

- Glomerular Filtration The glomerulus filters all nonprotein-bound drugs, whether lipid-soluble or insoluble, through bigger capillary holes. A drug's glomerular filtration is determined by its binding to plasma proteins and renal blood flow. The glomerular filtration rate (g.f.r.), typically around 120 ml/min, gradually decreases around the age of 50 and is low in renal failure.
- Tubular Reabsorption Passive diffusion relies on the drug's lipid solubility and ionization. Tubular Reabsorption Passive diffusion depends on the drug's lipid solubility and ionization at the urine pH level.
- Nonlipid-soluble and highly ionized medicines cannot diffuse in the tubules since 99% of glomerular filtrate is reabsorbed. However, lipid-soluble medications can. The rate of excretion of certain medications, such as aminoglycoside antibiotics and quaternary ammonium compounds, correlates with creatinine clearance.
- Urinary pH affects tubular reabsorption of partly ionized medicines. Weak bases are less reabsorbed in acidic urine, while weak acids are less reabsorbed in alkaline urine. This approach is used to facilitate drug elimination in poisoning by alkalizing the urine.
- In barbiturate and salicylate poisoning, urine is alkalized to help eliminate the medication. Although acidifying urine can improve the removal of weak bases like morphine and amphetamine, it is not recommended in clinical settings due to the risk of rhabdomyolysis, cardiotoxicity, and worsening outcomes.
- Changes in urine pH have the greatest impact on medication excretion in individuals with pKa values ranging from 5 to 8, as pH-dependent passive reabsorption is only significant in this range.
- Tubular secretion-The proximal tubules use nonspecific transporters (OAT and OCT) to actively transfer organic acids and bases.
- Additionally, efflux transporters P-gp and MRP2 are found in the luminal. Active drug transport across tubules reduces the concentration of free form in tubular capillaries and enhances dissociation of protein-bound drug, making it available for secretion.
- Protein binding, which can inhibit glomerular filtration, may actually facilitate tubular secretion.

a) OATP transports organic acids such as penicillin, probenecid, uric acid, salicylates, indomethacin, sulfinpyrazone, nitrofurantoin, methotrexate, and medication glucuronides/sulfates.

b) OCT transports organic bases such as thiazides, amiloride, triamterene, furosemide, quinine, procainamide, choline, and cimetidine. Both transport mechanisms are bidirectional, meaning they can move substrates from blood to tubular fluid and vice versa. However, regarding medications and their metabolites. For example, salicylates inhibit the uricosuric effect of probenecid and sulfinpyrazone while decreasing methotrexate tubular secretion.

- Probenecid reduces the concentration of nitrofurantoin in urine, prolongs the effect of penicillin/ampicillin, and affects methotrexate secretion.
- Sulfinpyrazone prevents the excretion of tolbutamide.

Kinetics of elimination

Provide a basis for dosage regimens and individual needs. Three fundamental pharmacokinetic parameters are bioavailability (F), volume of distribution (V), and clearance (CL). Drug elimination is the total of metabolic inactivation and excretion, primarily from the central compartment (blood) in equilibrium with peripheral compartments.

Plasma half life

The plasma half-life (t½) of a drug is the time it takes for its plasma concentration to decrease to half its original value. It is calculated from a semilog plasma concentration-time plot with two slopes: the initial rapidly declining phase due to distribution and the later less declined phase due to elimination. Most drugs have multicompartment distribution and multiexponential decay of plasma concentration-time plot. The elimination half-life is commonly mentioned and is calculated over the steady-state plasma concentration range. Drug clearance involves removing a fraction of the drug molecules per unit time.

The dose rate needed to achieve a target Cpss is determined by dividing the target Cpss by the concentration of the drug in the plasma. However, only a fraction of the dose reaches systemic circulation. The dose rate-Cpss relationship is linear only for drugs eliminated by first order kinetics, like phenytoin, which change from first to zero order kinetics over the therapeutic range.

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Abstract 2391

Novel HDAC Inhibitors as Potential Anticancer Drugs

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Gabriela Torres Quesada, Denisse Gutierrez, Armando Varela-Ramirez, Vijayalaxmi S, Subhas Karki, and Renato Aguilera

Keywords: Apoptosis, Anti-cancer drug discovery, Acute Lymphoblastic Leukemia, Cytotoxicity, HDAC Inhibitors

Introduction/background: Histone deacetylases (HDACs) are known to be overexpressed in many cancer types. This overexpression drives the proliferation of cancer cells and can repress tumor suppressor genes such as p53 and p21. HDAC inhibitors are relatively new in the field of cancer research, with some already approved by the Food and Drug Administration (FDA) to treat cancer patients, and others currently ongoing clinical trials. In this study, we evaluated the anticancer activity of seven novel HDAC inhibitors received from our collaborator, which included VS-169B, VS-183A, VS-183D, VS-186A, VS-186B, VS-186C, VS-186E, and compared them to their controls Curcumin and 5-fluorouracil. Experiments were conducted using two Acute Lymphoblastic Leukemia (ALL) cell lines, CEM and Jurkat, and one non-cancerous cell line, HS-27. The objective of this study is to see if these HDAC inhibitors display any cytotoxic activity, and if so, compare the activity in two human lymphoma cell lines. We also want to identify the mechanism of action that these drugs use to induce cell death, as well as find a drug treatment that targets cancer cells with minimal cytotoxicity to normal cells. Materials and Methods: The Differential Nuclear Staining (DNS) Assay was performed by growing the cells in flasks, plating them in 96 well plates, treating them with various concentrations of the drugs, incubating them for 72 hours, and reading them using a bioimager multi-well plate reader (Molecular Devices ImageXpress Pico). This instrument generates data that is used to calculate the CC50, or concentration that kills fifty percent of the cells. Flow Cytometry was used to check cell viability and perform assays such as the Annexin V FITC Assay, which determines the mechanism of action that the drug uses to induce cell death, whether that be apoptosis or necrosis. Results: All HDAC inhibitors displayed some anticancer activity. The compound VS-186B showed to have the most cytotoxic activity, with a CC50 of 2.9uM in the Jurkat cell line, and 4.6uM in the CEM cell line. The compounds displayed more cytotoxic activity than their controls Curcumin, and 5-fluorouracil. VS-186B was also tested on the human fibroblast HS-27 cell line to observe the cytotoxicity of the drug against non-cancerous cells. VS-186B exhibited a high selective cytotoxic index of 16.09 against Jurkat and 10.17 against CEM. Conclusion: Our cellular and molecular analyses indicate that the HDAC inhibitors displayed anti-cancer activity, with VS-186B being the most cytotoxic. Annexin V FITC analysis verified that this compound induced cell death by apoptosis. The high selective cytotoxic indexes confirmed that VS-186B was cytotoxic to cancer cells with minimal cytotoxicity to non-cancerous cells. We are currently using VS-186B in other assays such as mitochondrial depolarization, reactive oxygen species, and cell cycle analysis. Future experiments could include RNAseq gene expression analysis to compare the expression of tumor suppressor genes with and without HDAC inhibitor treatment.

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Abstract 2394

New Cyclic peptides Aigepatharitides A-C from an undescribed Irish deep-sea black coral Antipatharia spp

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Sam Afoullouss, Fred Valeriote, Louise Allcock, Mark Johnson, and Bill Baker

Keywords: Peptides, Anti-cancer, Natural Products, NMR, Black Coral

Shallow water black corals were harvested as long ago as the ancient Greek civilization, for which they were used as medicines and jewelry. More recently, international conservation measures have been enacted to preserve black corals and their habitats due to overharvesting. Medicinal usage of black corals may stem from their need for chemical defenses to withstand predation since they are unable to withdraw their polyps. With the aim of discovering novel anti-cancer drugs and increase conservational awareness of these organisms, deep-sea black corals from the Irish continental margin were screened for cytotoxicity against solid tumors. This bioassay-guided approach revealed several Antipatharia spp. with selective cytotoxicity against HepG2 cell lines. A chemical investigation of an undescribed Antipatharia sp. yielded three new cyclic peptides (aigepatharitides A-C) bearing the non-canonical amino acid dehydrotryptophan. Structure analysis and bioactivity of the metabolites will be discussed.

Funding sources include National Institutes of Health grant AT010939, Science Foundation Ireland (SFI) and the Marine Institute under the Investigators Programme Grant No. SFI/15/1A/3100, and European Regional Development Fund 2014-2020 NMBLI Grant-Aid Agreement PBA/MB/16/01

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Abstract 2396

Novel Ligase 1 and 4 Inhibitor Compounds as Potential Drugs for Cancer Treatment

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Keywords: apoptosis, anti-cancer, cytotoxicity, DNA ligase inhibitors, acute lymphoblastic leukemia

Introduction: DNA Ligases 1 and 4 play major roles in DNA replication. It has been shown that alterations in any of these may promote cellular proliferation in different types of cancer, such as Triple Negative Breast cancer and Ovarian cancer, due to the higher probability of DNA damage that they produce. Data has suggested that Ligase 1 and 4 inhibitors might be potential contributors for future cancer treatments. In this study, we evaluated the anticancer activity of five novel Ligase 1 inhibitors, which included PMJ-08, PMJ-09, PMJ-10, PMJ-11, PMJ-12 and PMJ-13, and five Ligase 4 inhibitors including PMJ-14, PMJ-15, PMJ-16, PMJ-17 and PMJ-18, all of them received from our collaborator. Experiments were conducted using CEM and JURKAT cell lines and compared to the non-cancerous control cell line, HS27. The goal of this study was to find which of these compounds have cytotoxic activity and identify the mechanisms of action of the most cytotoxic ones via further studies. Materials and Methods: A Differential Nuclear Staining Assay was made for each of the compounds with CEM and JURKAT cell lines after checking that cell viability was ∼90% in the Flow Cytometer. Cells were plated in a 96-well plate, treated with the different compounds and incubated for 48 hours. Results were obtained with the multi-well plate reader ImageXpress Pico and used to get the CC50's at 48 hours. The JC-1 mitochondria depolarization assay at 5 hours and Annexin V-FITC assay at 24 hours were made for compound PMJ-18. Results: Compounds PMJ-10 and PMJ-12 were insoluble. Compounds PMJ-08 and PMJ-09 precipitated at concentrations higher than 5μM and 10μM, respectively. Compound PMJ-11 showed exponential cell proliferation at higher concentrations. Compounds PMJ-09 and PMJ-16 did not show cytotoxicity. The most cytotoxic compound was PMJ-18 with a CC50 of 9.23μM. Phosphatidylserine (PS) externalization, a key feature of apoptosis, was examined using the Annexin V FITC Assay that indicated significant PS externalization. In addition, mitochondrial depolarization was observed using the JC-1 assay, again indicating activation of the intrinsic apoptosis pathway. Conclusion: Results of Annexin V FITC and JC-1 assays suggest that PMJ-18 kills cells via apoptosis. We are currently using PMJ-18 in the Reactive Oxygen Species (ROS) Assay to determine if ROS is generated after treatment. Future experiments could include RNAseq expression analyses to determine the mode of action of the compounds.

This research was funded by U-RISE at the University of Texas at El Paso from the National Institute of Health (NIH).

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Exploring the Therapeutic Potential of Murrayakoenigii in Letrozole-Induced Polycystic Ovary Syndrome: A Network Pharmacology and Experimental Study

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Background: Polycystic ovary syndrome (PCOS) is a heterogeneous endocrine and metabolic disorder associated with irregular menstrual cycles, dyslipidemia, excessive body weight, oxidative stress, hyperandrogenism, and insulin resistance. Effective management of PCOS remains elusive.

Objective: This study investigates the potential of Murrayakoenigii (curry leaves) as an herbal remedy in a rat model of letrozole-induced PCOS.

Materials and Methods: Thirty female Wistar rats were divided into five groups, with PCOS induced by letrozole administration (1mg/kg) for 21 days. Treatment groups received metformin (2mg/100gm) as a positive control or Murrayakoenigii extract at 400mg/kg or 800mg/kg for 15 days.

Results and Discussion: In-silico study revealed that the 7 phytocompounds of Murrayakoenigii leaves like Murrayacin, Alpha-carotene, Beta-Carotene, Bisabolol, Mahanimbinol, Gamma-Eudesmol, Lysophosphatidyl-choline showed action on P2RX7, EGFR, CNR1,

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HRH4, GABRA1, PLAU, PTGS2, FLT1, PRSS1, KDR, GRM5, PTGS1 genes though P13K-Akt, MAPK, VEGF, NF-kappa B, Arachidonic acid, Ras and Rap signaling pathways and EGFR tyrosine kinase inhibitor resistance pathway and so on might be responsible for managing the risk associated with PCOS. Murrayakoenigii treatment significantly reversed the effects of letrozole, including irregular estrous cycles, increased body and ovarian weight, dyslipidemia, oxidative stress, insulin resistance, and elevated testosterone levels. Network pharmacology analysis identified seven Murrayakoenigii compounds targeting genes and pathways associated with PCOS pathogenesis, suggesting potential mechanisms of action.

Conclusion: This study provides evidence for the therapeutic potential of Murrayakoenigii in managing PCOS, highlighting its importance as a natural alternative for PCOS treatment.

Keywords: Polycystic ovary syndrome, Murrayakoenigii, Network pharmacology, estrous cycles

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Study of neuroprotective effect of methanolic root extract of Moringa Concanensis against Cisplatin-induced peripheral neuropathy in rats

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Background: Peripheral neuropathy (PN) is a complex condition marked by damage to peripheral nerves, often exacerbated by chemotherapy, notably cisplatin. Current treatments for PN have limitations. *Moringa Concanensis* (MC), known for its medicinal properties, lacks scientific evidence for its efficacy against cisplatin induced PN. This study aims to explore MCs potential in rat models of PN to alleviate PN symptoms and improve cancer patient's quality of life.
Objective: Investigation of Methanolic root extract of *Moringa concanensis*(MC) as a potential treatment for Cisplatin-Induced Peripheral Neuropathy in rats.
Materials and Methods: Male Albino Wistar rats were divided into 5 groups: Normal Control, Positive Control, Low dose MC root extract (200 mg/kg.,p.o.), High dose MC root extract (400 mg/kg.,p.o.), Standard (Ascorbic acid) (45mg/kg.,p.o.). Cisplatin (2.5mg/kg.,i.p.) was administered in the second week and continued for 4 weeks, while MC root extract and ascorbic acid was administered orally from the first week for 5 weeks. After 5 weeks, Behavioural assessments and antioxidant activity in sciatic nerve homogenate was performed. Histopathological analysis of sciatic nerve samples was analysed using H&E stain.
Results: Treatment with methanolic root extract of MC at low dose and high dose for 5 weeks significantly improved behavioural parameters, antioxidant levels, attenuated oxidative stress and restored histopathological deterioration of sciatic nerve compared to the positive control group. However, grip strength and motor coordination of animals treated with standard showed no significant difference when compared to high dose.
Conclusion: The study indicates that methanolic root extract of MC protects Cisplatin-Induced Peripheral

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LIST OF PUBLISHERS NATIONAL/ INTERNATIONAL PROCEEDINGS FOR THE CONFERENCE DURING YEAR 2024

Sl.no	UGC-CARE ID	Publication Type	Publication Title	Author-name	Journal-name	Year
1	Non indexed	National	Inter-method Reliability of Glasgow Outcome Scale-Extended (GOS-E) - A Cross- Sectional Study	Dr. Komal K B , Dr. Brinda Dcosta	Scientific Research Journal of India	2024
2	Non indexed	National	Validation of HALO Digital Goniometer in Assessment of Range of Motion of Lumbar Spine in Healthy Individuals	Dr. Brinda Dcosta, Dr. Komal K B	Scientific Research Journal of India	2024

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Scientific Research Journal of India

Research Abstract

Inter-method Reliability of Glasgow Outcome Scale- Extended (GOS-E) - A Cross-Sectional Study

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BACKGROUND

Traumatic Brain Injury (TBI) is sudden damage to the brain caused when an outside force strikes the brain. After the discharge of TBI patients from the hospital, the functional status and disability of the patient remains unknown since most of the patients do not report or visit the hospital for timely follow up. The Glasgow Outcome Scale - Extended (GOS-E) is routinely used to describe outcome after TBI. There are two ways to administer GOS-E. When patient resides far from the healthcare facilities, face to face interview is not feasible especially in Indian scenario; telephonic interview method becomes an alternative. Moreover telephonic interview aids for large-scale investigations and also avoids observer bias. There is no literature on reliability of telephonic methods for examining GOS-E in Indian scenario. Hence the need arises to determine inter method reliability of Glasgow Outcome Scale Extended (GOS-E) in TBI survivors using telephonic method and face to face interview.

OBJECTIVE OF THE STUDY

This study investigated the inter-method reliability of Glasgow Outcome Scale Extended (GOS-E) in TBI survivors after three months using face to face interview and telephone method.

METHODOLOGY

Total of fifteen patients were recruited based on inclusion criteria at three months and later since the injury. Two raters were involved in this study,: the primary rater conducted face to face interviews at an outpatient clinic using the Kannada version of GOS-E, and a second rater, who had received training on GOS-E administration, conducted telephone interviews within six to eight hours.

RESULTS

TBI is more prevalent among the mean age 34.5 ± 11.8. There is excellent inter-rater reliability between GOS-E face to face interview and GOS-E telephone method, ICC = 0.95 at a 95% confidence interval.

CONCLUSION

Inter-method reliability of GOS-E in TBI survivors using face to face interview and on telephonic method has excellent reliability.

Note: *This research/article was presented at Kashi PhysioCon 2024, Varanasi, U.P., India.*

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Research Abstract

Validation of HALO Digital Goniometer in Assessment of Range of Motion of Lumbar Spine in Healthy Individuals

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BACKGROUND

Accurate measurement of lumbar spine range of motion (ROM) is essential in both clinical and research settings to assess spinal deficits, monitor progress in rehabilitation, and prepare treatment plans for various musculoskeletal conditions. The advent novel ROM assessment technology such as smartphone applications, gyroscopes, and devices like the HALO digital goniometer (HALO DG) have emerged as potential alternatives, offering convenience and enhanced precision in measuring joint movement across different anatomical regions. While research has validated the effectiveness of HALO DG in assessing range of motion for joints such as the shoulder, knee, and cervical spine, there is still a significant gap which exists in the literature with regards to its use for lumbar range of motion measurement. The lack of validation studies raises concerns regarding the accuracy and reliability of the HALO DG, particularly in the context of lumbar spine examination. Hence the major objective of this study is to address this research gap by evaluating the concurrent validity and intra-rater reliability of the HALO DG in contrast to the gold standard device, the double inclinometer (DI), for evaluating lumbar range of motion. This study seeks to give useful insights into the HALO DG's potential use in clinical practice and research, therefore contributing to the advancement of evidence-based methods in musculoskeletal evaluation, which will benefit both patients and healthcare professionals.

METHODOLOGY

The study included a convenience sample of 53 healthy subjects who met the inclusion criteria. Active lumbar flexion, extension, rotation, lateral flexion was measured. The evaluator measured each motion

thrice with each device (HALO DG and the DI) with due consent. The validity and reliability analysis were done using IBM SPSS Statistics version 25.

RESULTS

Pearson correlation coefficient was calculated to assess concurrent validity, strong positive correlations were observed across various movements, with correlation coefficients (r) of 0.97, 0.98, 0.94, 0.96, 0.98, and 0.98 for lumbar flexion, extension, right lateral flexion, left lateral flexion, right lumbar rotation, and left lumbar rotation, respectively. Furthermore, intra-rater reliability was assessed using the Intraclass Correlation Coefficient (ICC), the HALO DG (ICCm =0.99, 0.99, 0.98, 0.95, 0.99, 0.98) performed better than the DI (ICCm =0.97, 0.97, 0.95, 0.96, 0.98, 0.98) in lumbar flexion, extension, right lateral flexion, left lateral flexion and right lumbar rotation and left lumbar rotation respectively.

CONCLUSION

This validation study demonstrated that the HALO DG is a reliable and valid alternative for assessing lumbar range.

Note: *This research/article was presented at Kashi PhysioCon 2024, Varanasi, U.P., India.*

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Polymers for Pharmaceutical and Biomedical Applications
Fundamentals, Selection, and Preparation

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Chapter 3 - Characterization of polymers

John Disouza [a], Popat Kumbhar [a], Arehalli Manjappa [a b], Sameer Nadaf [c], Vijay Kumbar [d], Sachin Kumar Singh [e f], Kamal Dua [f g h], Vandana Patravale [i]

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Abstract

Polymers are the finest matrices for pharmaceutical and biomedical applications owing to their adaptability and customizable properties. The aptness of polymers for different applications is mainly related to their features. Consequently, it is desirable to characterize them for various attributes, including molecular weight, rheology, thermal behavior, etc. Polymer characterization with a suitable technique not only offers information on their properties and functionality but also aids in understanding the polymer qualities and adjustments required to optimize their performance. This chapter discusses solid state, solution state, and biological characteristics of polymers and techniques used for the investigation of the same. Additionally, methods to assess biodegradability and biocompatibility are covered. Other recent polymer characterization techniques are also described. Conclusively, investigating polymer properties using a suitable approach can contribute to significant advancement in exploring the applicability of newly developed polymers in various sectors such as engineering, pharmaceuticals, and biomedicine.

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