Theory and Applications of Nonparenteral Nanomedicines

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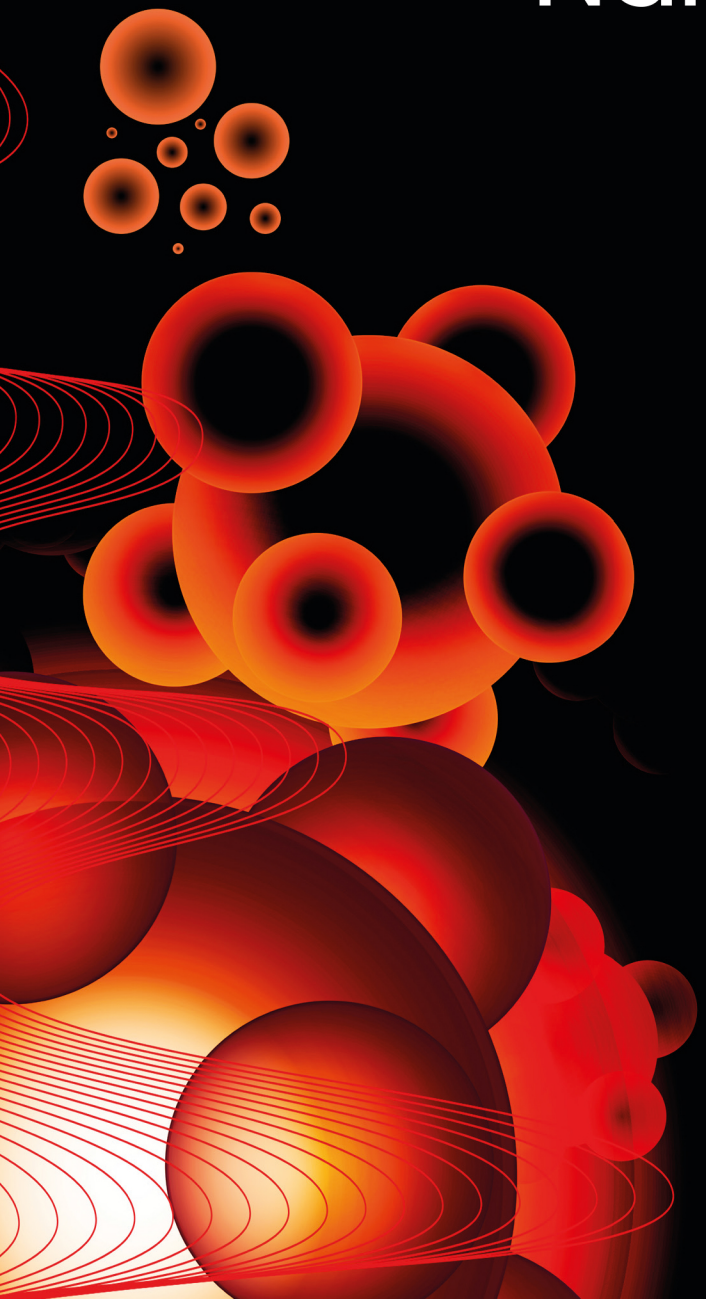

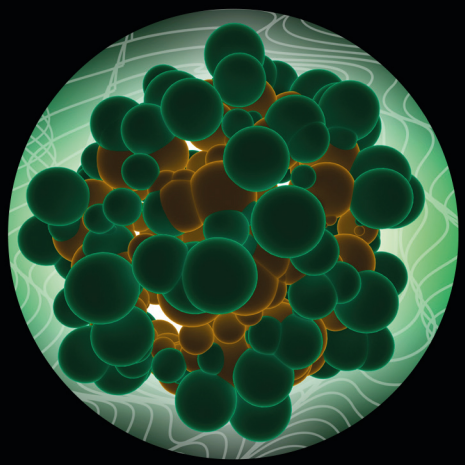

THEORY AND APPLICATIONS OF NONPARENTERAL NANOMEDICINES

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THEORY AND APPLICATIONS OF NONPARENTERAL NANOMEDICINES

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Dedication

I would like to dedicate this book to my parents (Mr. Hariom Kesharwani and Mrs. Anguri Kesharwani), my elder sister Dr. Poonam, and my younger brother Er. Pankaj, who always encouraged me throughout this journey. Equally, the book is dedicated to the love and sacrifices of my wife Garima, my sweet daughter Yashsavi, my nephew Adhyayan, and finally my mentor Professor N.K. Jain for believing in me and always being there for me.

Prashant Kesharwani

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Preface

The delivery of specific therapeutic agents to targeted cells is a hopeful prospect of nanotechnology in personalized medicine. This approach not only provides a specific treatment opportunity but also reduces the toxicity to the neighboring healthy cells. Nonetheless, nonparenteral routes offer multiple advantages compared to parenteral routes such as simpler needleless processes, shorter hospitalization, and overall better patient compliance. In recent years, multiple nanomedicines have been developed and optimized to bypass various physiological barriers and improve the delivery of poorly soluble drugs. The nonparenteral delivery of nanomedicine is still in its infancy. Further advances toward developing reliable nonparenteral nanomedicine will require the integration of different fields of biomedicine in the near future.

This book will provide a brief introduction to the nonparenteral delivery of nanomedicine, the safety, and regulatory implications of the nanoformulations. In further chapters, we will be discussing the physiology of the biological barriers, the specificity of the nanocarriers as well as their multiple applications. This book will help the readers to understand the recent progress in the design and development of nanoformulations compatibles with nonparenteral applications.

Focusing on the nonparenteral delivery of nanomedicine, this book will be a valuable resource for graduates, postgraduate, clinical researchers, and anyone working in the development of various nonparenteral-based nano-drug delivery system. Thus, this book explores a wide range of promising approaches for nonparenteral nanomedicine delivery. In totality, this book will prove to be one of the most comprehensive for the nonparenteral application of nanomedicine.

We thank all the authors for their valuable and timely contributions. This book brings pioneers of the field and early career scientists together, which is no doubt an indication of the commitment toward the advancement of nonparenteral therapeutics, to be continued across the generations to come to combat the most dreadful disease faced by humankind.

Prashant Kesharwani
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Editors

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CHAPTER 1

Introduction: An overview of the non-parenteral delivery of nanomedicine

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1. Introduction

The path by which a xenobiotic and/or drug reaches into the body is called the route of administration in the pharmaceutical sciences and is categorized according to the location at which the chemical is implemented [1]. For instance, intravenous, intramuscular, and oral administrations could be mentioned. In another classification, the routs are demonstrated based on the target where the substance acts. Enteral, topical, or parenteral administrations are considered to be in this category [1, 2]. Drug delivery is dependent on the route of administration and dosage. Among the numerous strategies pursued to optimize the physicochemical and pharmaceutic of drugs, the presence of a mucus layer that coats the surface of different organs has been capitalized to develop mucoadhesive dosage forms that remain in the administration site for more prolonged times, increasing the local and/or systemic bioavailability of the administered drug [3, 4].

As in many other fields, nanotechnology has gained increasing momentum in medical sciences offering distinct advantages. In this context, the application of nanoparticles as well as nanomedicine technology by means of nano-robots has opened new avenues in the field [5–7]. The groundbreaking progress of nanotechnology has endowed remarkable benefits to scientists in order to detect and treat a wide range of human diseases. The emergence of nanotechnologies together with the application of noninvasive and painless administration routes has altered the pharmaceutical science and the treatment of disease [8, 9].

Parenteral administration has been mainly used to deliver nanotherapeutics in the market. In addition, non-parenteral routes such as nasal, dermal, ocular, and pulmonary pathways are the expected target of nanotherapeutics in the near future [10]. It is important to select the best delivery route and pass the plausible barrier to have an efficient drug

Theory and Applications of Nonparenteral Nanomedicines
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delivery system. It should be able to protect the drugs against degradation, improve therapeutic effect, extend biological activity, control drug release rate and performance, and decrease the frequency of administration [11]. Furthermore, an efficient drug delivery system must provide protection to human body in which it is administered. These features could be attained by using a safe material to prepare carriers of drugs like different types of biodegradable polymers [12]. Chitosan as a cationic polymer of natural origin is an outstanding instance of an excipient that currently has vast potential for use in pharmaceutical dosage forms. It is a polyelectrolyte with reactive functional groups with ability to form gel and include high adsorption volume. Biodegradability, biocompatibility as well as being nontoxic to living tissues together with its antibacterial, antifungal, and antitumor properties make it to an outstanding biomaterial. These properties offer suitability and extensive pharmaceutical applications particularly proposed to be administered via non-parenteral routes (oral, topical, intranasal, vaginal, rectal, and ocular) [13–15].

This chapter provides a state-of-the-art review on the present non-parenteral delivery of nanotherapeutics with focus on technologies that have utmost pharmaceutical and commercial capabilities. Mucoadhesive systems and chitosan-based non-parenteral nano delivery systems are at the core of attention in this chapter due to their importance in non-parenteral delivery of nanomedicines.

2. Routes of administration

The exposition location usually determines the route of administration. Pharmacokinetics is concerned with the path by which the substance is transferred from the application location to its target site and involves the uptake, distribution, and elimination processes. Transdermal or transmucosal routes are considered as exceptions. On the contrary, physiological effects of the active substances at the location of the target effect are called pharmacodynamics [6, 16–18]. However, topical administration is an exception since both the application and the target effect location are the same and is occasionally referred to as a local application location and local pharmacodynamics impact. But, sometimes it is called a local application location irrespective of the location of the effects [19–21].

2.1 Enteral/gastrointestinal administration

Enteral or enteric which means through intestines is an administration route through gastrointestinal tract which also includes oral and rectal administrations [22]. Although oral and rectal administrated drugs are uptaken by intestine, orally prescribed drug might also be received by stomach. Moreover, some application locations such as buccal, sublingual, and sublabial are considered as enteral administration routes without reaching the intestines. Thus, gastrointestinal is a more comprehensive term and best fits with this route of administration. There are also particular enteral administrations such as in the contrast enema, whereby the contrast media is directly infused into the intestines. However,

the term enteral is utilized for chemicals with systemic effects when the classification is based on the site of the effects [23, 24].

Some drugs are injected into a gastric tube while tablets, capsules, and drops are taken thorough mouth. Gastric feeding tubes or gastrostomy methods facilitate direct administration of medications into the stomach. Also, duodenal feeding tubes and enteral nutrition and approaches as well as enteric-coated tablets deliver the substances into the small intestine [25].

Many drugs especially aging medications are administrated via the rectal route where a rapid and effective absorption occurs through the walls of the rectum [26]. The absorption of medications from the distal one-third of the rectum bypasses the first-pass effect through the liver resulting in a high bioavailability of drugs in comparison with oral administration [27]. Moreover, the highly vascularized rectal mucosal provides a quick and efficient absorption system. The solid dosage form of drug that is used for rectal administration is called a suppository. Rectal catheters are also available in clinical care to provide a discreet and simple delivery route [28].

2.2 Topical

When both the application location and the pharmacodynamics effect thereof are local, the term topical administration is utilized. Also, topical route is defined as delivery of a substance to the surface of body unrelated to the location of the effect [29]. Transdermal route by which the drug is administrated onto the skin but induces systemic effects is considered a topical route. Enteral administration of drugs that are poorly absorbed through the gastrointestinal tract could also be classified as a topical route [30].

2.3 Parenteral and non-parenteral routes

In parenteral route, drug is administered into the space between gastrointestinal tract and body surface. This administration method is divided into particular routes such as IV, IM, SC, ID, IA, IP, etc. Parenteral administration is classified into four categories: intramuscular, intravenous, subcutaneous, and intradermal injections. Any route that is not enteral is considered a parenteral. A needle and a syringe or an indwelling catheter is implanted to perform a parenteral administration [31, 32]. Parenteral administrations could be injected into the following locations:

- Eperidural injection in central nervous system and epidural in which the drug is delivered to the epidural space as in anesthetic agents [33].
- Intracerebral injection which directly delivers the substances into the cerebrum and is utilized in empirical studies and treatment of brain tumors [34].
- Intracerebroventricular injection which delivers the drug into the brain ventricles as in the delivery of opioids in end-stage cancer patients [35].

- Epicutaneous injection which delivers the drug into the skin tissue. Allergy tests of several drugs could be mentioned in this classification [36].
- Buccal and sublingual administration in which the drug is placed between gum and cheek and under the tongue, respectively. Steroids, cardiovascular drugs, vitamins, and minerals in different drug forms are prescribed via this route [37].
- Extra-amniotic injection that delivers the drug into the space between fetal membrane and the uterus [38].
- Nasal administration that delivers topical drugs or insufflations through the nose [39].
- Intra-arterial injection which is particularly used to deliver vasodilators or thrombolytic agents into the arteries [40].
- Intra-articular injection is used to inject drugs into the space of joint which is appropriate for osteoarthritis treatment [41].
- Intracardiac injection (directly into the heart muscles or ventricles) is not a commonly used method for the injection of drugs like adrenaline in cardiopulmonary resuscitation [42].
- Intracavernous injection or penile injection in the treatment of erectile dysfunction [43].
- Intradermal injection is suitable for performing skin testing for some allergens and for the diagnosis of tuberculosis [44].
- Intralesional administration is used for the delivery of drug into skin lesions locally [45].
- Intramuscular administration which is a common injection method for muscle delivery of many medications such as vaccines, antibiotics, e.g., Ref. [46].
- Intraocular administration that delivers glaucoma or eye neoplasms medications into the eye [47].
- Intraosseous infusion is used for the delivery of emergency medicine and pediatrics into the bone marrow in specific situations that intravenous access is not possible [48].
- Intraperitoneal administration which is particularly used to deliver drug into the peritoneum or in peritoneal dialysis [49].
- Intrathecal administration which directly delivers the substances into the spinal canal [50]
- Intravaginal administration that delivers the drug into the vagina [51].
- Intravenous administration is commonly used for the delivery of many drug forms into a vein [52].
- Intravesical infusion that delivers drugs into the urinary bladder [53].
- Subcutaneous administration is used commonly to deliver specific drugs like insulin under the skin [54].
- Transdermal administration which is particularly used to diffuse transdermal patches such as fentanyl (relief of pain), nicotine (treatment of addiction), and nitroglycerine (for angina pectoris) [30]

- Perivascular administration that deliver drugs locally around a blood vessel in open vascular surgery [55].
- Transmucosal administration which is particularly used to diffuse substances via mucous membrane route; snorting (cocaine), under the tongue (nitroglycerine), and vaginal suppositories [56].

In non-parenteral administration, drug is given by the mouth or applied to the skin surface (topical administration, inhalation, or aerosol administration) [57]. Table 1 presents the summary of the characteristics for parenteral route versus non-parenteral routes.

Table 1 The summary of the characteristics for parenteral route vs non-parenteral routes.

Parenteral route	Non-parenteral routes
• Any route that is not enteral is considered as parenteral • Parental administrations are classified in four types: intramuscular, intravenous, subcutaneous, and intradermal injections • A needle and a syringe or an indwelling catheter is implanted to perform a parenteral administration	• In non-parenteral administration, drug is given into the mouth or skin surface (topical administration, Inhalation, or aerosol administration) [57] • Oral route which is a non-parenteral administration of drugs into the mouth • Ear drops which is a non-parenteral administration route directly delivers the drugs into the war canal in order to pain or infection treatment [58] • Eye drops which is particularly used to drug delivery of dilating drugs, glaucoma and infection medicines [59]. • Inhalation is commonly used to direct delivery of drugs into nose and mouth [60] • Rectal route which is particularly used to directly deliver suppositories or solution form of drugs into rectal cavity [61] • Sublingual or buccal as non-parenteral administration routes are used to delivery of drugs into lip or tongue or between the cheek and gums [62] • Topical route which directly deliver the ointment, cream, or lotion form of substances into the skin or mucous membrane [63] • Vaginal route is a common non-parenteral route in the treatment of yeast infection or other irritations in vagina [64]

3. Nanotechnology, nanomaterial, and nanomedicine

Nanotechnology is defined as the design and use of nanosized structures (1–100 nm), although researchers in different fields have slightly modified it. Nontechnology discusses about the materials which have one dimension that measures particle sizes less than 100 nm.

Medical application of nanotechnology has brought up unique possibilities. While some techniques are still a part of human imagination, most of them are at different phases of testing, or are utilized as the current novel methodologies. Nanotechnology in medical fields is applied in two forms: the use of nanoparticles and nano-robots, which are utilized to repair organ dysfunction at cellular level.

Delivery of drugs, light, heat, and other substances to the target cells is the useful application of nanotechnology in biomedicine. The proper engineering of the nanoparticles enable them to have the ability to enter the diseased cells directly. This approach not only provides a specific treatment opportunity but also protects the neighboring healthy cells. In an investigation conducted in North Carolina State University, a novel method has been developed to deliver cardiac stem cells to the injured heart tissue via the application of nano-vesicles that have a high affinity to the damaged heart. This increases the number of stem cells attracted to the injured tissue. Carbon nanotube-attached antibodies have been used to detect cancerous cell in peripheral blood. This technique provides a rapid and simple test system in cancer detection. Gold nono-rods have been implemented to attach proteins released in kidney damages. This detection platform diagnoses kidney damages in early stages in a rapid and cost-effective way. The accumulation of the above-mentioned proteins changes the color of nono-rods. Gold nanoparticles have also been used to eliminate bacteria together with infrared light which can be beneficial in antiseptic aspects in hospital settings. Quantum dots have also been effective in the reduction of antibiotic-resistant infections.

In the recent years, nanotechnology and nanobiotechnology have gained importance in medicine and medical technology. Nanomedicine owns the great potentials to meaningfully advance the quality of life of patients or even healthy persons [6, 65, 66]. Fig. 1 shows the research and development zones which received the greatest motivation from nanomedicine in the recent decades.

4. Mucoadhesive systems in pharmaceutical research and drug delivery area

The main purpose of pharmaceutical research is to make products with confirmed quality to capably treat disease. One of the challenges in the pharmaceutical science is the poor aqueous solubility and permeability of drugs. This physicochemical property is common to approximately 50% of the active pharmaceutical ingredient (APIs) on the market and

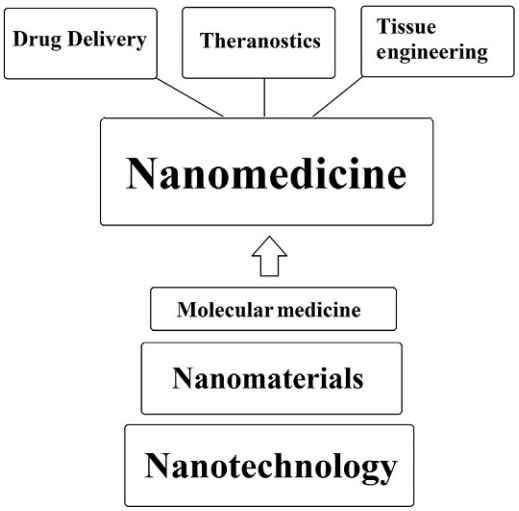

Fig. 1 Development areas of nanomedicine.

represents a crucial hurdle during the stages of drug product development. Moreover, low solubility in biological fluids makes limited absorption in the gastrointestinal tract (GIT) and imperfect bioavailability, the oral route being the most popular one.

Solubility is an intrinsic property that depends on the nature of the molecule, whereas dissolution is an extrinsic one that can be modified by different means such as reduction of drug particle size and encapsulation in a variety of micro and nanocarriers [67, 68]. Among the different approaches followed to enhance the physicochemical and pharmaceutic platforms of drugs, the presence of a mucus layer that coats the surface of different organs has been capitalized to develop mucoadhesive dosage forms that remain in the administration site for more prolonged times, increasing the local and/or systemic bioavailability of the administered drug [31]. The appearance of nanotechnologies together with the application of noninvasive and painless administration routes has changed the pharmaceutical science.

4.1 Molecular structures of mucosae

Mucosae, and in particular mucosal fluids, are an essential part of mucoadhesive phenomena. Mucosal tissues cover natural body cavities, providing an epithelial barrier to the external environment. One important issue has been to deal with shearing at mucosal sites [31, 69, 70].

The mucosae is defined by the presence of a protective layer of fluid namely mucus, which acts as a physical barrier against chemical and biological agents, as well as a natural lubricant. This fluid also has important homeostatic roles, namely, clearing cellular debris, regulating water balance and ion transport, mucosal immune-regulation, transporting

sperm in the cervicovaginal tract, among others. Mucus is produced in mucous glands and by goblet cells in the airways at the mucosa/submucosa, except in the case of the stomach (mucus is produced by epithelial cells) and the vagina. Vaginal lubrication is a naturally produced fluid that lubricates a woman's vagina, which results from the mixture of different liquids including mucus formed at the cervix. Mucus is a non-Newtonian viscoelastic fluid, which is secreted in a concentrated form and then undergoes hydration into a gel-like mesh. The width of the mesh spaces delimited by mucin fibers has been formerly estimated to be around 20–200 nm [31, 69–71].

4.2 Mucoadhesion of nanosystems

Nanoparticle size, shape, surface chemistry, and composition are all key criteria which influence nanosystem behavior in mucoadhesive systems. These properties of the materials are tunable over orders of interactions with mucus fluids present at various mucosae [72]. Since mucoadhesion implies attachment to the buccal mucosa, films can be achieved by using mucoadhesive polymers as matrix-forming materials, alone or in mixtures. However, not all the available mucoadhesive polymers can be easily applied to produce nanoparticles or, for instance, other nonpolymeric systems may be desirable (e.g., lipid-based nanosystems). In the last case, the attaching mucoadhesive polymers on preformed nanosystems (covalently or by simple adsorption) or conjugating polymers with other matrix-forming materials might be an alternative method for surface modification. Surface charge of nanocarriers is another important parameter; positively charged systems are preferred in order to maximize mucoadhesion [31]. Chitosan-based nanoparticles, in particular, is often cited as an example of the "highly mucoadhesive nanosystem." Also, hydrophobicity of nanosystems can also affect the ability of adhesive interactions with hydrophobic domains of mucin, namely, by promoting hydrophobic bonding [31, 69, 73].

4.3 Mucoadhesive systems for non-parenteral delivery of nanomedicines

Three main groups of polymers are used in the preparation of mucoadhesive systems: natural (alginate, chitosan and its derivatives, guar gum, xanthan gum and pectin, galactomannan and glucomannan, carrageenan κ type II 6-hyaluronic acid and other glycosaminoglycans, gelatin), synthetic polymers [Poly(ethylene glycol), poly(ethylene oxide), poly(acrylic acid), poly(methacrylic acid) derivatives, poly(vinyl pyrrolidone), poly(vinyl amine), boronate containing polymers], and semisynthetic polymers (cellulose derivatives) [69, 70].

This section introduces each non-parenteral administration route and the main therapeutic advantages of making a system mucoadhesive.

4.3.1 Oral administration

Oral administration is the most practical approach for drug delivery because of superior patient compliance, especially in the therapy of chronic maladies because its methodology included noninvasive and painless procedures and enables self-administration. These points are of special interest for the treatment of pediatric patients [70]. The field of mucosal administration has been led by the development of drug delivery systems for oral administration. However, to achieve this aim, nanocarriers need to be appropriately caused to display mucoadhesive features. Nanosuspensions produced by the dispersion of pure drug nanoparticles of hydrophobic drugs by bottom-up or top-down methods are probably the simplest nanotechnology applied to pharmaceutical sciences and is included mainly to enhance dissolution rates and, by doing so, to advance bioavailability [74].

4.3.2 Inhalatory administration

The airways provide for fast absorption and large surface area that can be advantageous in the treatment of pulmonary obstructive diseases (e.g., asthma) and overcoming local infectious diseases due to the limit of systemic contact and adverse effects and also for the systemic delivery of drugs in the so-called transpulmonary route. At the same time, the potential of this alternative way has promoted the novel aerosol technological development that the administered dose and the removal level. Inhalant drugs must have a number of properties that include aerosol particles with mean aerodynamic diameter between 0.5 and 5 μm to favor deposition in the deep lung, aerosol particles with low size distribution and high reproducibility, dissolution or adhesion adhesive to the mucosa, and suitable drug release and permeability [75, 76].

4.3.3 Ocular administration

The eye is a complex organ consisting of several functional and mutually interacting parts. The sclera is made of fibrous tissues shaped as segments of two spheres, the sclera and the cornea. From a medical point of view, cornea and conjunctiva represent two major mucosal barriers so proper drug permeability makes it possible to cross the barrier and to reach the regions of a drug's action. The cornea is a dense, avascular connective tissue constituting 90% of the cornea thickness and providing the mechanical integrity and transparency to the cornea; nutrients and oxygen are provided to the cornea by the lachrymal and aqueous fluids [77]. The most posterior layer of the lid is the conjunctiva, a thin transparent vascularized mucous membrane that covers the pericorneal surface and the posterior, which lines the inner surface of the eyelids and is reflected onto the globe [78].

4.3.4 Vaginal administration

The vagina has been traditionally used as an advantageous site for drug delivery and constitutes an interesting route (if not preferential in some cases) for the management of local gynecological conditions to achieve either local or systemic effects [31]. Moreover,

owing to the good absorption profile of some compounds through its mucosa, vaginal administration may be performed to obtain systemic drug levels that have been shown useful for hormonal contraception or replacement therapy, inducement or prevention of labor, and pregnancy termination. In the last decade, huge interest has been focused on the investigation in the field of vaginal drug therapy, mostly because of the development of microbicides [31, 79].

4.3.5 Intranasal administration

The nose is a highly complex organ that accomplishes a great variety of functions that range from olfaction to humidification, warming, and filtering of the inhaled air before it reaches the trachea and the lungs. The nasal mucosa contains two layers, the underlying lamina propria that is rich in blood and lymphatic vessels, nerves, glands, and cells of the immune system and the luminal epithelium containing goblet cells that produce the mucus that covers the epithelium. The nasal mucosa is a site for local and systemic drug delivery because of a highly vascularized epithelium mucosa, a relatively large surface area, the high irrigation, and the presence of lymphocytes and mast cells [80].

4.3.6 Buccal/sublingual administration

Buccal drug delivery includes the administration of the desired drug through the buccal mucosal membrane lining of the oral cavity. A sublingual tablet formulation with an enhanced acceptability has been successfully developed where the drug is released and undergoes fast absorption into the systemic circulation [81]. The rich irrigation flow, thinner mucosa with respect to other body sites (the sublingual being even thinner than the buccal) and increased permeability, limited enzymatic activity, and ability to develop unidirectional release systems that minimize oral absorption are added advantages. Thus, very high drug concentration levels can be achieved without gastric or hepatic metabolism. On the other hand, the drug can be partially swallowed which causes a decrease in bioavailability and a delayed concentration peak [31].

5. Chitosan-based nanomaterials for drug delivery purposes

An efficient and safe drug carrier must provide protection to human tissues and/or organs in which it is administered as well as protection to the drugs against degradation, control drug release rate, prolong biological effect, improve therapeutic activity, and reduce the frequency of administration.

These benefits could be obtained by the development of drug carriers using natural polymers like chitosan. Chitosan is an abundant cationic polysaccharide of natural origin and is derived from the chitin that is found in the cell walls of certain fungi, algae, and bacteria species, as well as in the shells of mollusks and the exoskeleton of the phylum Arthropoda [82–84]. Generally, chitosan is obtained by the alkaline deacetylation of

chitin at different thermochemical conditions or by enzymatic hydrolysis using a chitin deacetylase [85, 86]. Chitosan is almost the only cationic polymer in nature which currently holds enormous promise for use in pharmaceutical dosage forms because of its unique features such as polyelectrolyte containing reactive functional groups, high capacity for adsorption, gel-forming capability, biodegradable and biocompatible to living cells or tissue as well as having antifungal, antibacterial, and antitumor effects [73, 87]. These substantial characteristics offer suitability for various pharmaceutical applications such as regenerative medicine for many components such as growth factors, proteins/peptides, antibiotics, anti-inflammatory drugs intended for non-parenteral use (intranasal, oral, topical, ocular, etc.) and for the development of drug carriers (drug conjugate, hydrogels, emulsions, micro/nanoparticles, etc.). The latest development on nanotechnology and the various functionalization processes of chitosan have improved its functionality as drug delivery system and developed systems more versatile by using chitosan in combination with other components in novel systems. Here, we review advantages and limitations of chitosan-based carrier systems to improve the delivery of drugs through the non-parenteral routes.

5.1 Chitosan-based nanomaterials for non-parenteral drug delivery

In recent years, a considerable amount of research work has been focused on chitosan-based vehicles and their potential use for drug delivery through noninvasive routes, such as mucosal (nasal, oral, ocular, and vaginal) and (trans) dermal. The primary amino groups of chitosan are responsible for properties such as in situ gelation, mucoadhesion, permeation enhancement, transfection, controlled drug release, and efflux pump inhibitory effects. The mucoadhesive characteristics are also attributed to its cationic character. Mucoadhesion can be obtained through the ionic interactions between the cationic primary amino groups of chitosan and anionic substructures of the mucus gel layer due to the presence of sialic acid and sulfonic acid substructures. Moreover, hydrophobic interactions can contribute to its mucoadhesion characteristics. Chitosan has also been utilized in a wide range of pharmaceutical multipurpose excipients to enhance water solubility and increase stability of drugs [88].

5.1.1 Nasal delivery systems

Overall, the absorption of drugs via nasal mucosa is often comparatively poor due to a short local retention time, low membrane permeability, and high turnover rate of a secretion in the nasal cavity [89]. Chitosan-based particles or polyelectrolyte complexes have been widely investigated as promising materials for intranasal delivery of therapeutic proteins [90, 91]. It was reported that chitosan nanoparticles loaded with insulin promote nasal absorption of drug to a greater extent as compared with the aqueous chitosan solution. Additionally, many studies have used chitosan in nasal vaccines as a potent mucosal adjuvant. In a recent study, nasal drip using chitosan in combination with the matrix protein 1

was used as adjuvant for immunization (twice at an interval of 3 weeks) of BALB/c mice. According to the results, the adjuvant chitosan effectively improved the vaccine efficacy and could provide effective protection for mice against the homologous virus (H9N2) [92]. Chitosan-based microparticles and gels with different solubility and molecular weights have also been shown as adjuvant/delivery system for nasal immunization against bovine herpes virus 1 (BHV-1) [93]. Notably, morphology and appearance were observed to be similar (spheroids with a smooth surface) in blank microparticles formulated using base chitosan. It was found that the surface appearance of microparticles changed as the chitosan molecular weight increased. This might contribute to the entrapment of antigens within the microparticles, as well as adsorption of antigens on surface of the microparticles [94]. Therefore, these systems are promising mucosal adjuvant-delivery systems tested for noninvasive delivery of antigen [95, 96].

5.1.2 Oral delivery systems

In recent decades, chitosan-based formulations have been extensively studied for the delivery of pharmaceutical agents to specific body sites such as oral cavity, stomach, small intestine, and large intestine. The site-specific drug delivery to the oral cavity can be utilized to treat mouth diseases such as stomatitis, fungal and viral infections, periodontal disease, and oral cavity cancers, thereby preventing the first-pass effect. Nevertheless, salivary flow and buccal motion could reduce the mucosal absorption. Consequently, the dosage form must show good mucoadhesion capability to maintain the device in its position for many hours and to have an efficient control of drug delivery. It was reported that drug release is affected by matrix swelling and erosion, whereas matrix adhesiveness can be modulated by appropriate selection of the polymers or polymer mixtures, both adhesive and not. Chitosan has excellent mucoadhesive property and a significant enhancement effect on the penetration of drugs through the buccal mucosa [97, 98]. The outstanding characteristics of chitosan microspheres support their wide applicability for systemic as well as for local therapy. For instance, thermosensitive hydrogel containing chlorhexidine-loaded chitosan microparticles demonstrated a potent antibacterial effect of chitosan or as an activator for the antibacterial process [99]. The use of antibiotic-loaded microspheres have also been proved to treat gastrointestinal diseases such as peptic ulcer [99], intestinal infections, ulcerative colitis, and carcinomas [100]. On the other hand, it has been reported that chitosan-based formulations can be superior in improving absorption of therapeutic peptides and proteins as well as induction of antibodies following mucosal vaccination [101, 102]. For example, an appropriate selection of chitosan level in coating thickness could reduce drug release and provide zero-order release in a medium of simulated intestinal fluid. The microcrystalline cellulose core beads loaded with 5-aminosalicylic acid were coated with Aquacoat ECD and chitosan mixtures to achieve controlled release of drugs in the small and large intestines.

These beads were reported to be susceptible to the enzymatic action of rat cecal and colonic bacteria and exhibited high potential for colonic drug delivery [103].

5.1.3 Ocular delivery systems

Over recent years, various ophthalmic carrier systems such as ointment, suspensions, aqueous gels, and inserts have been studied to prolong the residence time of the dose instilled to stay on the surface of the eye and improve the ophthalmic bioavailability. Among these, in situ forming gels have been demonstrated as good candidates to prolong the precorneal resident time and to improve ocular drug bioavailability. Therefore, chitosan-based vehicles have ability to increase the retention and biodistribution of topically applied drugs onto the eye [104, 105]. Mucoadhesive chitosan nanoparticles have been shown to be a good drug delivery system for ocular mucosa. For example, fluorescence-labeled chitosan nanoparticles were attached to the cornea and the conjunctiva for at least 24 h [106]. Besides, chitosan-based colloidal systems exhibited promising results as transmucosal drug vehicles, by mediating drug transport to the inner eye and increasing their accumulation into the corneal epithelia. The use of chitosan-based colloidal suspensions in vivo showed a significant increase in ocular drug bioavailability. According to in vivo results, chitosan-based colloidal suspensions exhibited a significant improvement in ocular bioavailability of drug [107]. Similarly, bioadhesive chitosan microspheres were studied for ocular delivery, in which a high concentration of acyclovir was obtained over a prolonged period of time through in vivo studies on rabbits. In addition to its mucoadhesive features, authors investigated that chitosan is effective in retarding drug release rate [107]. In another study, in vitro and in vivo biocompatibility and cytotoxicity data show that chitosan microparticles were effective to achieve long-term drug or protein delivery systems to the outer segment of the retina. Chitosan microparticles have a high encapsulation efficiency and a sustained release profile as compared with the polyethylene glycol-polylactic acid (PEG–PLA) microparticles. However, the concentration of chitosan microparticles may be critical for assessing the toxic potential of particles, and the concentration required will depend on the appropriate amount of the drug (or protein) required to achieve a therapeutic dose and encapsulation capacity [108].

5.1.4 Topical/transdermal delivery systems

Transdermal drug delivery systems can transport pharmaceutical agents through skin layers for systemic effects at a controlled rate (can be interrupted if it is necessary), thereby avoiding the first-pass effect [109, 110]. For example, transdermal chitosan patches containing lidocaine hydrochloride were shown as a drug reservoir which release drug in a sustained profile at 95% chitosan degree of deacetylation [111]. In another study, chitosan nanoparticles loaded with warfarin-β-cyclodextrin were successfully prepared via ionic gelation technique. They were found to be spherical with a narrow size distribution

and exhibited a high drug entrapment efficiency. Here, chitosan nanoparticles have an initial burst effect followed by an extended period of slow continuous release. The results also demonstrated that nanoparticle formulation elevated the warfarin permeation through excised rat skin in a constant and continuous manner, offering a promising system for the transdermal delivery [112].

5.1.5 Regenerative systems

In order to regenerate a damaged or missing organ, in vitro seeding and attachment of human cells on a scaffold, followed by cell culture to form the new organ must to be performed [113]. Chitosan is known as one of the most highly exploited polymers for tissue and organ regeneration because of its biodegradability, biocompatibility, antibacterial, and wound-healing activities. Additionally, capacity of chitosan (unmodified or as a derivative) can help prepare efficient scaffolds having desirable features such as porous structure, gel forming properties, and high affinity to in vivo macromolecules [114]. On the other hand, chitosan has a structure quite similar to that of glycosaminoglycans which constitute the main component of the extracellular matrix. Many studies have addressed this issue by conjugating chitosan-based materials to cell adhesion moieties, thereby aiming to mimic functions of the extracellular matrix. Biodegradability of the scaffold material is also important and it is preferable to use the materials that are absorbed or eliminated from the body without the need for surgical removal. In this regard, the in vitro assay of the responsiveness of articular chondrocyte-like cells by a multimembrane chitosan hydrogel demonstrated that a high concentration of cartilage-type matrix proteins were produced [115]. Chitosan has been widely utilized in bone tissue engineering because of its ability to elevate cell growth and mineral-rich matrix deposition when used for osteoblast culture [116]. In the field of liver tissue engineering, hepatoma HepG2 cells were seeded onto microfluidic chitosan microfibers without chemical additives to treat acute and chronic liver disease. The data showed cells forming spheroids aggregates, with high liver function that was confirmed by urea synthesis and albumin secretion. This strategy exhibits a potentially appropriate tool for liver tissue engineering applications [117]. Table 2 presents some of the chitosan based non-parenteral delivery systems.

6. Non-parenteral routes for vaccination delivery

Since the introduction of vaccination centuries ago, it has been one of the most beneficial strategies to control infectious disease. It has effectively eliminated small pox, almost eradicated polio, and decreased the incidence of diphtheria, tetanus, and measles. Despite these advantages, the application of vaccines is controversial, and much of this debate is related to the use and safety of adjuvants in vaccines, which are necessary to improve vaccine efficacy. In the past decade, particularly, the application of nanotechnology in

Table 2 Some of the chitosan based non-parenteral delivery systems.

Formulation	Administration route	Advantage	Reference
Chitosan nanoparticle/ DNA complexes	Nasal	A potential system for the induction of both humoral and mucosal immune responses	[118]
Olanzapine loaded chitosan nanoparticles	Nasal	An appropriate treatment of depressant via olfactory nasal pathway to the brain	[119]
Chitosan/Calcium-Alginate beads	Oral	Desired controlled release of insulin for a convenient gastrointestinal tract delivery system	[120]
Alginate-coated chitosan microparticles	Oral	Protection of BSA from degradation in acidic medium in vitro and suitable for mucosal vaccine	[121]
Alginate-chitosan film	Ocular	Improved ocular gatifloxacin sesquihydrate bioavailability and patient compliance	[122]
Montmorillonite/ chitosan nanoparticles	Ocular	Increased retention time and bioavailability	[123]
Poloxamer/chitosan in situ forming gel	Ocular	Sustained release and high drug permeability as compared to solutions of the drug	[124]
Lidocaine chitosan gels	Topical	An increase in both the rate and extent and also in permeability of drug	[125]
Minoxidil sulfate-loaded chitosan microparticles	Topical	Sustained release and high drug permeability	[126]
Macroporous chitosan patch	Transdermal	Improved efficiency of cell adhesion and bioadhesive property	[127]
Rivastigmine-loaded chitosan microparticles	Transdermal	Increased retention time and skin permeability	[128]
Chitosan/ hydroxy propyl methyl cellulose polymer Blends	Transdermal	Good film forming property	[129]

vaccinology has been increasing exponentially, leading to the appearance of "nanovaccinology" [130]. In both therapeutic and prophylactic immunization approaches, nanoparticles are utilized as a delivery system to improve processing of antigen and/or as an immunostimulant adjuvant to induce or increase immunity. Therapeutic nanovaccinology is commonly used for the treatment of cancer [131–133], and is progressively explored for the treatment of other diseases or conditions, such as hypertension [134], Alzheimer disease [135], and addiction to nicotine [136]. On the other hand, prophylactic nanovaccinology has been used for the inhibition of different diseases [137].

There are two main delivery methods for the administration of vaccine, parenteral via intramuscular and subcutaneous, and non-parenteral vaccination via oral, intranasal, rectal, and vaginal routes [138]. To date, the common delivery routes for vaccination are parenteral routes, and there is much less investigation on non-parenteral vaccination [138]. Nonetheless, non-parenteral routes have beneficial advantages such as simpler needleless process and better patient compliance. For systemic and local delivery of vaccines, intranasal administration route is a favorable choice and has been shown to be effective in different animal models and humans [139]. Even then, its progress has been hindered by problems related to the necessity of delivery apparatuses such as nebulizers and showed neurotoxic side effects such as redirection of live-attenuated organisms or toxin-based adjuvants to the central nervous system upon intranasal immunization [140, 141]. The most generally used non-parenteral route is oral administration, which is capable of stimulating mucosal immune response in gastrointestinal tract, but nevertheless has some drawbacks such as antigen degradation by gastrointestinal enzymes, necessity of high doses, and effect of first-pass metabolism [142]. Advances in developing vaccines based on either oral delivery or intranasal have been hindered due to retraction of their live weakened vaccines as adverse effects were observed [139].

Seth et al. investigated the efficiency of polycaprolactone (PCL), poly(D, L-lactic-co-glycolic acid) (PLGA), and silica (Si-OH) nanoparticles (nps) to adjuvant recombinant capsomere presenting antigenic M2e modular peptide from influenza A virus (CapM2e) in vivo. Formulation of CapM2e with PLGA or Si-OH nps considerably enhanced the modular capsomeres immunogenicity, even though CapM2e was not actively attached to the nanoparticles prior to injection (i.e., formulation was prepared by simple mixing method). Nevertheless, PCL nps displayed no momentous adjuvant effect by means of this simple mixing method. The immune response stimulated by CapM2e alone or combined with nps was antibody-biased with very high titer of antigen specific antibody and less than 20 cells per million splenocytes secreting interferon gamma (IFN-γ). Modification in the properties of silica nanoparticle surface via pegylation and amine functionalization did not cause significant changes in response of immune system [143].

Khademi et al. reviewed the potential of different polymeric nanoparticles as future vaccine delivery systems/adjuvants for parenteral and non-parenteral (mucosal) immunization routes against tuberculosis (TB). According on their investigation, since the entrance site of TB is the respiratory mucosa, mucosal vaccination, particularly the nasal route, could provide good protection against TB [138, 144]. This could be achieved by the production of neutralizing antibodies like systemic IgG, secretory IgA (sIgA), and activation of different CD4+ T lymphocyte such as Th17, Th1, Th2, and CD8+ T cells (CTLs). These cells could strongly induce both systemic and mucosal immune responses [145, 146]. Th1-, Th17-, and CTL-mediated immunity is necessary for defense of the host body against intracellular microorganisms that enter via various mucosal surfaces, whereas CD4+ Th2 cells are effective against extracellular microorganisms [146–148].

Antibody responses via opsonization of microorganism and accordingly more efficient processing by dendritic cells (DCs) have synergistic effects on the immune system [149]. Moreover, the nasal cavity has other advantages such as better epithelial permeability, patient compliance, self-administration, and does not require needles [138, 150]. Their systematic review results shows that for the development of a better new TB vaccine, more attention to expressed antigens in early and latency phase of M. tuberculosis infection is required. On the other hand, co-delivery of TB vaccines with polymeric nanoparticles could be helpful for improving the weak immunogenicity of these antigens and decreasing the limitations in the development of novel and more efficient TB vaccines. Their study showed that variety of polymeric nanoparticles could be utilized as carriers for mucosal and parenteral administration of TB vaccine candidates. Among the polymers studied, PLGA and chitosan polymers are the most excellent nanoparticles that display promising results, after administration via subcutaneous and mucosal routes, respectively [144].

7. Summary, conclusion, and future perspectives

In this chapter, we have provided an overview of non-parenteral delivery of nanomaterials. The main focus was on technologies that have utmost pharmaceutical and commercial capabilities. Mucoadhesive systems and chitosan based non-parenteral nano delivery systems are at the core of attention in this chapter due to their importance in non-parenteral delivery of nanomedicines. Non-parenteral routes have beneficial advantages compared to parenteral routes such as simpler needleless process and better patient compliance. Delivery of drugs or other biomaterial substances to the target cells is one of the main features of nanotechnology in medicine (nanomedicine). The appropriate engineering of the nanomaterials enable them to have the ability to enter the diseased cells directly which not only provides a specific treatment option but also protects the neighboring healthy cells. The most important non-parenteral route is oral administration, which is capable of stimulating mucosal immune response in gastrointestinal tract, but nevertheless has some drawbacks such as antigen degradation by gastrointestinal enzymes, necessity of high doses, and effect of first-pass metabolism.

Mucosal tissues play a significant role in developing novel nano-based drug delivery systems. Mucoadhesive drug delivery systems have shown the ability to improve the bioavailability of drugs, diminish systemic exposure, and increase the therapeutic index. A wide range of natural or synthetic materials (polymers) exists for use in pharmaceutical areas to prepare mucoadhesive systems. However, owing to a lack of the variety in mucoadhesive systems, nanotechnology platforms have not been investigated properly. Therefore, mucoadhesive nano-based drug delivery systems have not been introduced in the market. Such a state exposes the problems faced to conduct clinical trials.

Our review process also shows that chitosan is almost the most important cationic polymer in nature which currently plays enormous promising role for use in pharmaceutical dosage forms owing to its unique properties. Its substantial features offer suitability for various pharmaceutical applications such as regenerative medicine for many components such as growth factors, proteins/peptides, antibiotics, anti-inflammatory drugs intended for non-parenteral use (intranasal, oral, topical, ocular, etc.), and for the development of drug carriers (drug conjugate, hydrogels, emulsions, micro-/nanoparticles, etc.). Numerous therapeutic agents, such as anticancer, anti-inflammatory, antibiotics, antithrombotic, steroids, proteins, amino acids, antidiabetic, and diuretics, have been loaded in chitosan-based nanosystems. These systems have shown improved dissolution rate for poorly soluble drugs together with controlled release performance. The hydroxyl and amino functional groups of chitosan lead to significant consequences and valuable physicochemical properties that improve the stability, and the mucoadhesively property which prolong the drug residence time of the drug delivery systems. Despite the vast number of reports about non-parenteral nano-based delivery systems of chitosan-based materials, more studies are needed to improve the mechanical properties of the prepared system. More toxicity tests also should be directed in order to confirm their safety.

There is much less investigation on non-parenteral vaccine delivery. Non-parenteral routes have helpful benefits for vaccine delivery such as simpler needleless process and better patient compliance. For non-parenteral delivery of vaccines, intranasal administration route using nanomaterials has shown a promising option and has been demonstrated to be effective in different animal models and humans. Even then, its development has been delayed by issues related to the necessity of delivery apparatuses such as nebulizers and presented neurotoxic side effects such as redirection of live-attenuated organisms or toxin-based adjuvants to the central nervous system upon intranasal immunization.

It can be concluded that advances in developing non-parenteral delivery systems for nanomedicine applications should be continued by investigators from different fields of biomedicine to achieve more advanced delivery systems. In this state, the future years will be vital to combine the arena and to report the novel pharmaceutical products with ideal features that will support the valuable influence of the nanomedicine (nanosystems) for treating disease.

Conflict of interest

The authors declare that they have no conflict of interest.

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CHAPTER 2

Challenges in nonparenteral nanomedicine therapy

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1. Therapeutic and theranostic nanomedicines: Introduction

Nanomedicine and its therapeutic potential are new but evolving science field, where nanoscale materials are employed as a tool for disease diagnosis or targeted drug delivery in a very precise manner. Targeted delivery of chemotherapeutic, immunotherapeutic, and biologic agents in treating numerous diseases is an outstanding application of nanomedicine. Therapeutics based on nanoparticles have great potential to influence the treatment of various human diseases, but instability and early release from nanoparticles decrease the bioavailability of drugs, which impedes its clinical translation. All nanoparticles must rely on control at the nano-size scale, which means small variations may cause significant changes to the nanoformulation [1]. Drug delivery with nanotechnology can offer greater control over the biodistribution of therapeutic agents and thus improve the overall therapeutic index. Researchers are focusing on customized nanoparticles at sizes and shapes complimentary to the biological entities that may act precisely during the cargo and on the actuation process. When it is optimized, such a method should greatly reduce the adversities and side effects, particularly that of chemotherapy, imparting to a patient's healthy cells. The goal of most nano-based strategies for drug delivery is to enhance the therapeutic effectiveness of the active pharmaceutical ingredient (API) and also to reduce the adverse effects [2]. The specificity of measuring API that was previously considered unimportant has now gained much importance due to the understanding of specific pharmacokinetic profiles and dose-limiting toxicity that are critical efficacy determining factors for viable therapies using nano-based delivery strategies. The past two decades have witnessed the unprecedented growth of nanomedicines that are translated into clinics as well. As nanomedicines evolved, techniques to properly evaluate their safety and efficacy are also evolved. Characterization methods for imaging and analysis of nano-based materials are also evolved in demand of the nanomedicine developers and regulators. Pharmacokinetic characteristics

Theory and Applications of Nonparenteral Nanomedicines
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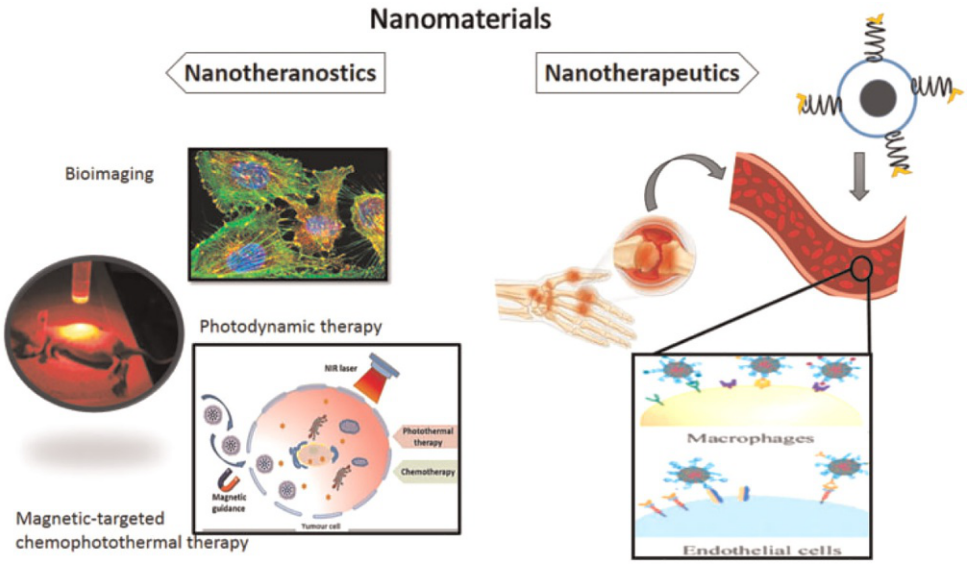

Fig. 1 An illustration of theranostic and therapeutic applications of nanomaterials. *(Adapted with permission from Naila Qamar, et al., Nanomedicine: an emerging era of theranostics and therapeutics for rheumatoid arthritis, Rheumatology 58 (10) 1715–1721. Copyright 2019 © Oxford University Press).*

of various nanomedicines with different formulations are determined by particle size, shape (chemical structure), and surface chemical characteristics [3]. The aim of regulating particle size in nanomedicines is to increase their retention in target tissues and to remove them rapidly when distributed to nontarget tissues. Nanomedicines with particle size less than 10 nm are removed by kidneys whereas those with particle size more than 10 nm are sometimes elongated and removed by the liver and/or the mononuclear-phagocyte system (MPS). The physicochemical properties of nanoparticles assist the binding of cellular, blood, and protein components that ease their interactions with immune cells eliciting the immune response [4]. Some developments were also made to synthesize conjugated nanomedicines like that are attached to physiological membranes (by the fusing immune cell membranes to polymeric cores) and thus have immense promise to suppress synovial inflammation, deactivate pro-inflammatory cytokines and provide strong chondroprotection against inflamed joints (Fig. 1).

1.1 History and advancement of nano-therapeutics

The first synthesis of therapeutic nanoparticles can be traced back to the 1950s when polyvinyl-pyrrolidone-mescaline conjugate was developed by incorporating a short peptide spacer between the drug and the polymer [5]. Another early influential event occurred in the mid-1960s when liposomes were discovered [6]. These discoveries mark the birth of the field of nano-therapeutics and during the recent past, relative innovations

of nanocarriers represent most of the highlighted therapeutics and continue to be investigated extensively. Nanoparticle targeting based on chemical properties of nanoparticles and surface coatings comprises active and passive targeting [7]. Passive targeting is defined as nonspecific accumulation in disease tissue (usually cancer tissue). Specific or active targeting is defined as selective transport of nanomedicines containing protein, antibody, or small molecule only to specific tissues and/or specific cells [8]. This may occur via homing to overexpressed cell-surface receptors (Fig. 2).

1.2 History and advancement of nanotheranostics

Theranostics, the coupling of therapeutic products with diagnostic agents, can provide feedback through imaging results or other diagnostic probes about the efficacy of treatment. This may help in optimization and personalization of treatment more efficiently than the current standard of care [9]. Theranostics usually refers to a combinatory scheme of diagnostic therapy for individual patients, testing them for possible reactions when taking a new medication and tailoring their treatment based on personalized test results. By adding the prefix nano, the term nanotheranostics appears where the role of nanomaterials in treatment delivery is dominant [10]. Nanotheranostic is a unique and unconventional treatment approach that carries immense potential to influence our health-care systems. From the last few years, various theranostic systems have been widely used for imaging, therapy, and development of targeted drug delivery systems toward various diseases and disorders [1, 11]. Besides imaging and therapy, nanotheranostic systems are being used to monitor pharmacokinetics, distribution of the particles in the tissue, and accumulation of drug at the target site, etc. However, a lot of factors limit their applications especially in the usage of such formulations as contrast agents and drugs because of not capable of entering the brain due to blood-brain barrier (BBB), which pose a major challenge in the path of development of an efficacious and safe theranostic system [12]. NPs which exhibit low toxicity profile hold great opportunities to be developed as nanotheranostic systems. A major challenge, in nanotheranostics, for the 21st century, is to be able to detect disease biomarkers noninvasively at an early stage of disease progression and its usage as personalized medicine for genetic and phenotypic disorders [13] (Fig. 3).

2. Designing nanomedicines for nonparental administration

Nanomedicines have evolved into various forms including dendrimers, nanocrystals, emulsions, liposomes, solid lipid nanoparticles, micelles, and polymeric nanoparticles since their first launch in the market [14, 15]. The creation of "smart" nanoparticles is an emerging trend in nanomedicine. To facilitate pharmacokinetic and biodistribution analysis, and to thereby improve drug targeting to pathological sites, it would be highly useful if the circulation time and the organ accumulation of nanomedicine formulations could be visualized noninvasively in real-time [16]. To achieve this goal, many different

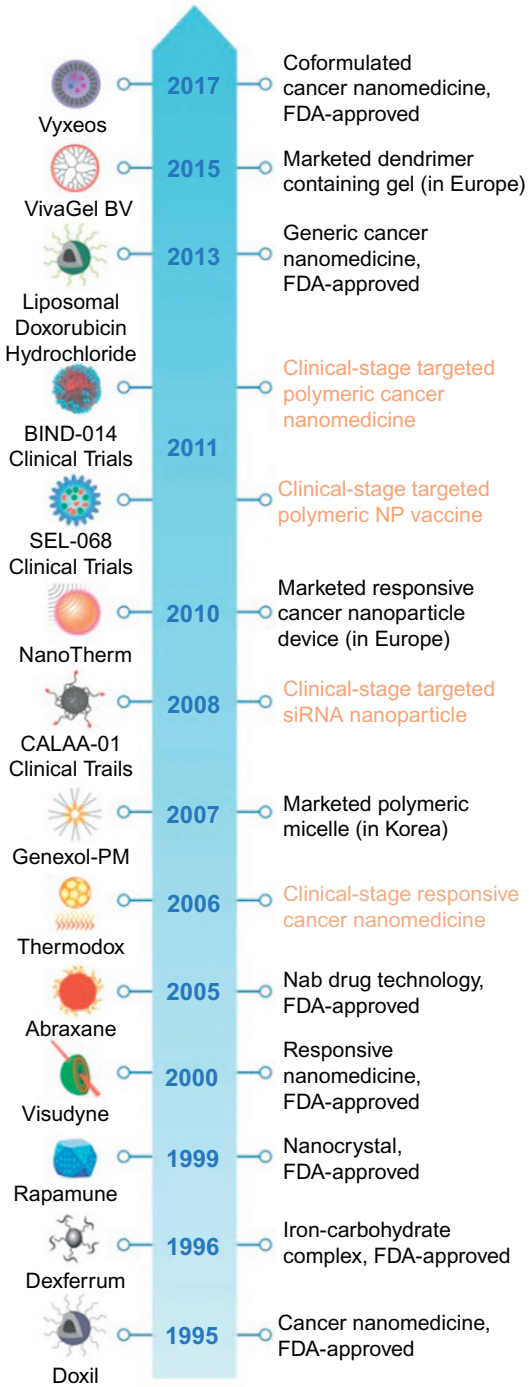

Fig. 2 The evolution of nanomedicines demonstrated with examples that are in clinical trials or reached the market. *(Adapted with permission from Swierczewska, et al., Characterization of nanoparticles intended for drug delivery, in: Methods in Molecular Biology, vol. 1682, 2018. Copyright 2018 © Springer).*

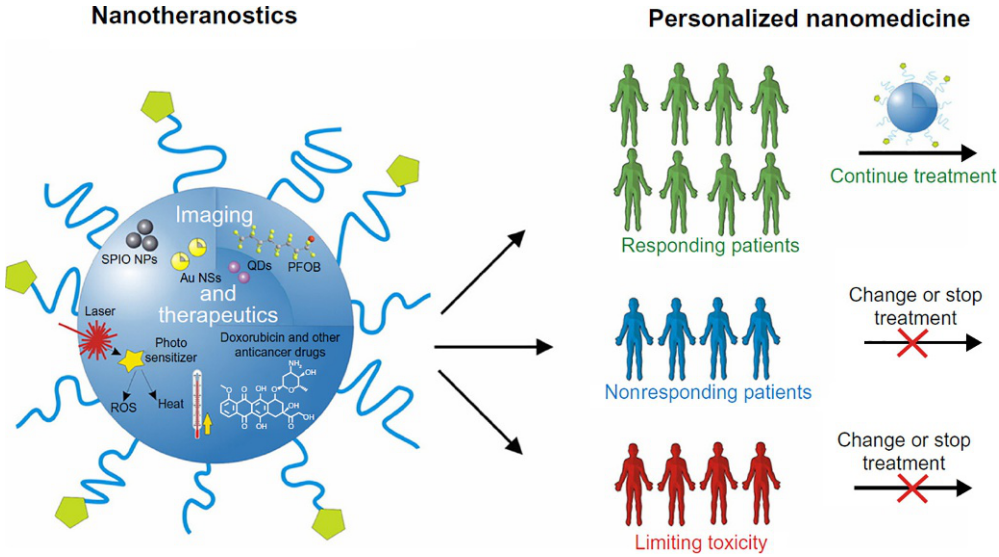

Fig. 3 Potential of nanotheranostic systems in personalizing treatment and improving therapeutic outcomes. *(Adapted with permission from S. Mura, P. Couvreur, Nanotheranostics for personalized medicine, Adv. Drug Deliv. Rev. 64 (2012) (13) 1394–1416. Copyright © Elsevier).*

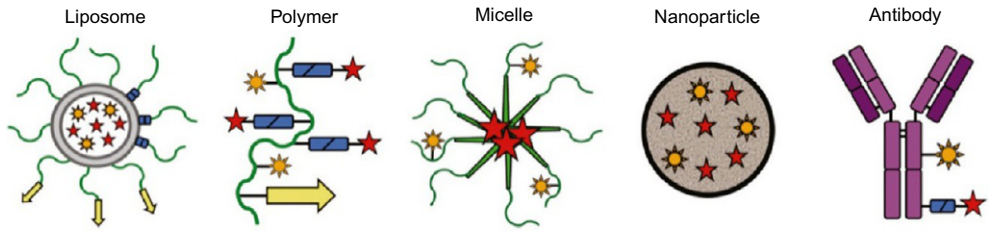

Fig. 4 Classical designs of nanomedicines conjugated or entrapped pharmacologically active agents. *(Adapted with permission from Lammers, et al., Theranostic nanomedicines and image-guided drug delivery, Acc. Chem. Res. 44 (2011) (10) 1029–1038).*

types of nanomedicines have been coloaded both with drugs and imaging agents. By delivering pharmacologically active agents more effectively and more selectively to the pathological site (site-specific drug delivery) and/or by guiding them away from potentially endangered healthy tissues (site-avoidance drug delivery), nanomedicines aim to improve the balance between the efficacy and the toxicity of systemic (chemo) therapeutic interventions [17, 18] (Fig. 4).

Due to the wide range use of polymeric biomaterials, a single, ideal polymer or polymeric family does not exist. Instead, a library of materials is available to researchers that

can be synthesized and engineered to best match the specifications of the material's desired biomedical function [19]. Since drug release patterns greatly vary from batch to batch of nanomedicine formulations, and since there are large differences in the release patterns, for example, liposomes vs polymers versus micelles, it is of the utmost importance to visualize and analyze drug release, not only under semiartificial in vitro conditions but also under physiologically relevant in vivo conditions [20, 21]. In vitro, drug release can generally be analyzed relatively easily, for example, using HPLC, but in vivo this is much more complicated: after harvesting the target tissue, for instance, the material generally needs to be homogenized, and the cells need to be lysed, in order to release the agents from certain intracellular compartments [22]. During these processing steps, and especially during cell lysis (using detergents), many types of carrier materials are destabilized, and, for example, in the case of liposomes, it is then impossible to discriminate between the amount of drug that was still present within liposomes at the point of harvesting and the amount that was already released into the extra- and intracellular environment [23, 24]. The opsonization of intravenously administered nanoparticles decreases their circulation time, thus affecting the drug delivery efficacy of nanomedicine at the inflamed site [25]. The disturbed vasculature in the inflamed joints is also a limiting factor. The most remarkable quality of nanostructures is the engineered capability to carry substances of choice; they can be functionalized more biocompatible by appropriate designing procedures [26]. As a result of this freedom, researchers have been able to develop more targeted, biocompatible, and biodegradable nanomedicines, which is a step toward providing a sustainable solution to the long-standing ailments. The novel nanotheranostic and nanotherapeutic strategies being researched not only retain the potential to specifically target inflammation sites but could also reduce the dose and administration frequency of drugs to a minimum. Nanoparticles are composed of inorganic or organic material and are of diameter 1–100 nm; they exhibit novel and unique properties as compared with bulk materials but also exhibit considerable toxicity because of their high reactivity with chemicals, increased cell permeability, and their large surface area, and inner pore dimensions [27]. Liposomes are lipid vesicles that are composed of phospholipids, cholesterol, and other lipid conjugated polymers with an inner aqueous phase. The liposomes can load hydrophilic drugs in the inner aqueous core and lipophilic drugs in the lipid bilayers. Polymeric NPs can be engineered to load a high content of drugs and provide controlled drug release for prolonged periods of time. Dendrimers are globular, nanostructured polymers with a well-defined shape and narrow polydispersity (3–20 nm). Drugs could be either entrapped in the dendrimer core or conjugated to the dendrimer surface functional groups [28]. The drug-loading capacity and drug-release profile of dendrimers can be controlled by the dendrimer generation, surface chemistry and conjugation method. Micelles are self-assembled spherical vesicles consisting of hydrophilic corona and a hydrophobic core, which shows the potential to solubilize and stabilize hydrophobic drugs [29] (Fig. 5).

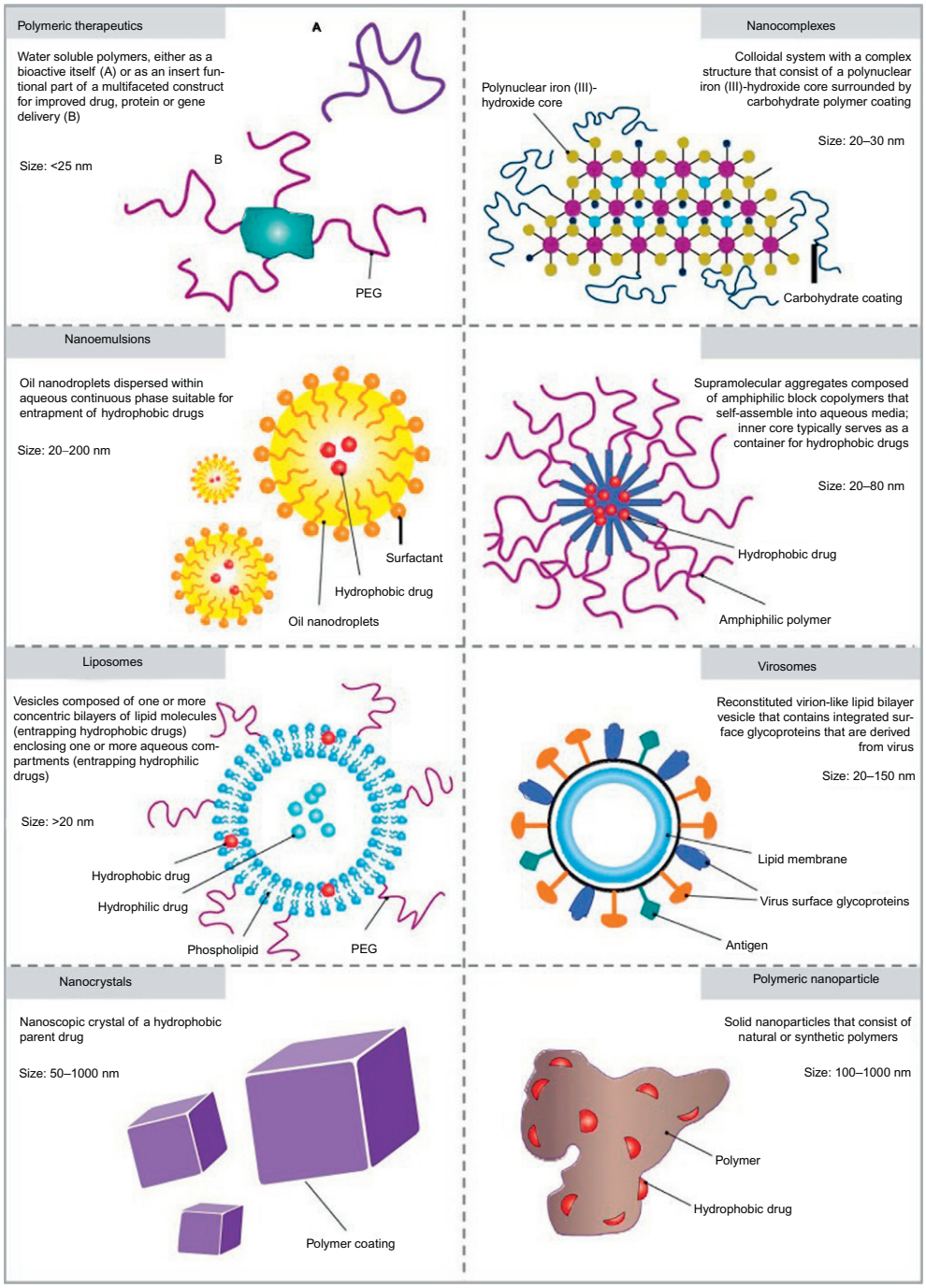

Fig. 5 A schematic overview of nanotherapeutic formulations with their respective size averages. *(Adapted with permission from A. Hafner, et al., Nanotherapeutics in the EU: an overview on current state and future directions, Int. J. Nanomedicine 19 (2014) (9) 1005–23. Copyright © 2014 Dove Medical Press Limited).*

2.1 Nanomedicines with natural polymers

Biopolymer nanoparticles can be used efficaciously to provide bioactive molecules for in vivo and in vitro applications. Nano-biopolymers also find applications in the field of enzyme replacement therapy (ERT). The emergence of stimuli-responsive polymeric systems and polymer-drug conjugates has greatly influenced the rational design of polymers tailored for specific cargo and engineered to exert distinct biological functions [30, 31]. Indeed, the possibility of using nanotherapeutic agents constituted by biocompatible and biodegradable polymers to deliver enzymes in those tissues where they are lacking or absent represents an enormous advantage by overcoming a series of ERT problems [32]. Natural polymeric nanomedicines are proved to be effective in stabilizing and protecting biologically active components, including vaccines, DNA, proteins, etc., from various environmental hazards and degradation. It was demonstrated that natural polymer-based nanomedicines have enhanced therapeutic efficacy as a result of the prolonged systemic circulation, targeted drug delivery, and cellular uptake [33, 34]. For example, alginate NPs proved to be good delivery vehicles for vaccine adjuvants, such that they stabilize and protect antigens from the immediate biological environment, slow down antigen clearance, and enhance delivery to antigen-presenting cells, especially dendritic cells [35] (Fig. 6).

During the recent years, preparation and processing of natural products-based nanomedicines are considered as the promising scientific arena because they have interesting characteristics, such as being biodegradable, biocompatible, being renewable with better drug availability, and also exhibiting very less toxicity compared to conventional pharmaceutical candidates [36]. The engineering of new polymeric derivatives that are

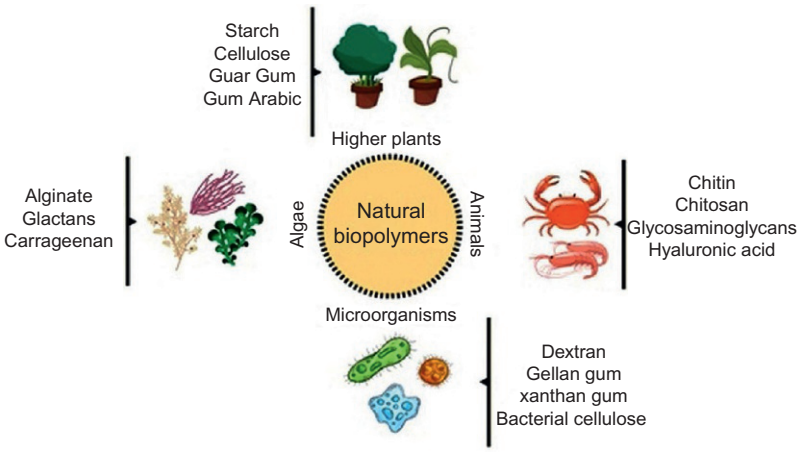

Fig. 6 Various biological sources from which natural biopolymers can be extracted and used in nanomedicine applications. *(Adapted with permission from Patra, et al., Nano-based Drug delivery systems, J. Nanobiotechnol. 16 (2018) 71).*

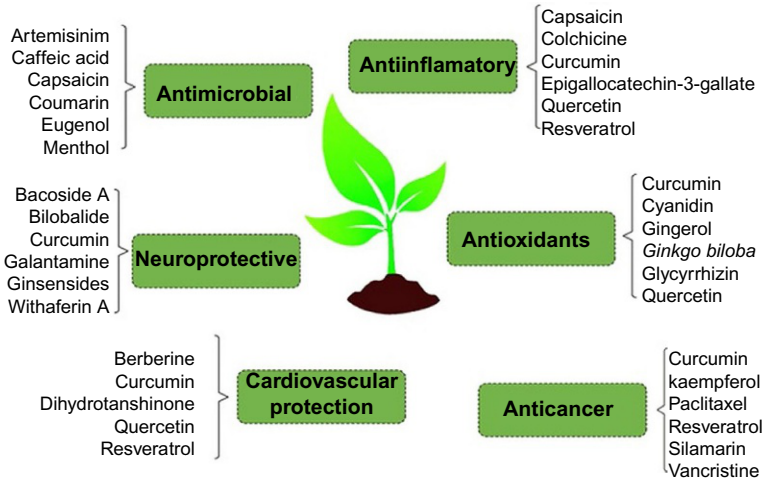

Fig. 7 Several ethno-pharmaceutical compounds were identified and extracted from herbs and higher plants which are used widely for nanotherapeutic applications. *(Adapted with permission from Patra, et al., J. Nanobiotechnol. 16 (2018) 71).*

capable of drug release by endogenous or exogenous stimuli has been introduced during the last 10 years and continue to be investigated [37]. These stimuli-responsive mechanisms are based on pH change, ionic strength change, enzyme-substrate interaction, magnetic stimuli, thermal change, electrical, and ultrasound stimuli. Stimuli induce changes in the surrounding environment that affect polymer physical and chemical proprieties [38] (Fig. 7).

Biodegradable polymers (synthetic, semisynthetic, and natural) used for the development of NPs possess unique characteristics including nanoscaled structures, high encapsulation capacity, biocompatibility, and controlled-/sustained-release profile for lipophilic/hydrophilic drugs. Despite the tremendous effort that was made to enhance natural polymeric nanocarrier properties, still some limitations can be observed, manifested by their poor drug-loading capacity, drug expulsion after polymeric transition during storage, and the tendency for particle-particle aggregation as a result of their large surface area [39].

2.2 Nanomedicines with synthetic polymers

There are various synthetic biodegradable polymers such as poly(hydroxylbutyrate), poly anhydride copolymers, poly(orthoester)s, polyphosphazenes, poly(amidoester)s, poly(cyano acrylate)s, and PLGA. PLGA is a widely used polymer that has been approved by the US Food and Drug Administration (FDA) for various therapeutic/diagnostic applications [40]. Principally, drug release from polymeric nanomedicine involves the movement of a drug molecule from the initial position in the polymeric matrix to the

polymer's outer surface and finally into the surrounding environment. It should be noted that PLGA undergoes hydrolytic degradation in an aqueous environment where ester linkages along with the polymer backbone are randomly hydrolyzed. Drug release may occur via one or a combination of the following mechanisms: diffusion, dissolution, degradation, or swelling [41]. Generally, if drug diffusion across the polymeric matrix is faster than matrix degradation, then the mechanism of drug release is driven mainly by diffusion, otherwise, polymer degradation is the limiting step in drug release. Consequently, drug release normally follows first- (via matrix degradation) rather than zero-order (via diffusion) kinetics [42]. Particle size also strongly influences drug release through mediating both diffusion and matrix degradation. Drug release from a synthetic polymeric nano-formulations is also highly influenced by desorption of the surface-bound/adsorbed drug by diffusion and erosion [43]. Rapid initial or burst release can be attributed to the fraction of the drug that is adsorbed or weakly bound to the large surface of the polymeric nanocarriers (NCs), rather than drug molecules incorporated in the NCs itself (Fig. 8).

Hence, recent efforts to design and develop biodegradable polymeric nanomedicines have been focused on custom designing and synthesizing polymers with tailored properties for specific applications by (i) developing novel synthetic polymers with unique chemistries to increase the diversity of polymer structure, (ii) developing biosynthetic processes to form biomimetic polymer structures, and (iii) adopting combinatorial and computational approaches in biomaterial design to accelerate the discovery of novel resorbable polymers [44].

2.3 Nanomedicines with multifunctional adaptations

The morphological and chemical modifications of natural polymers produce semisynthetic polymers that are better suited for processing and production of materials with potential of mineralization and conversion to biomass. To improve the applicability of such semisynthetic polymeric forms and its various derivatives (e.g., carboxylated, thiolated, and acylated structures) for pharmaceutical/biomedical applications, they have so far been decorated with various functional groups such as polyelectrolyte/polyionic complexes [45]. Biodegradable polymeric micelles composed of PEG and polycarbonate functionalized with disulfide and carboxylic group can be synthesized as pH and redox dual responsive drug delivery systems [46]. Hydrophilic thermosensitive biodegradable polymeric nanocarriers, are another example of smart drug delivery systems that are collapsed at the hyperthermic condition of 42 °C which causes greater drug release and may lead to a synergistic effect of chemotherapy and hyperthermia for treatment of solid tumors [47]. Furthermore, the biodegradable polymeric carriers have been modified by tumor-targeting agents such as specific ligands (e.g., folic acid), antibodies and aptamers to enhance the nanomedicine translocation into tumor cells [48] (Fig. 9).

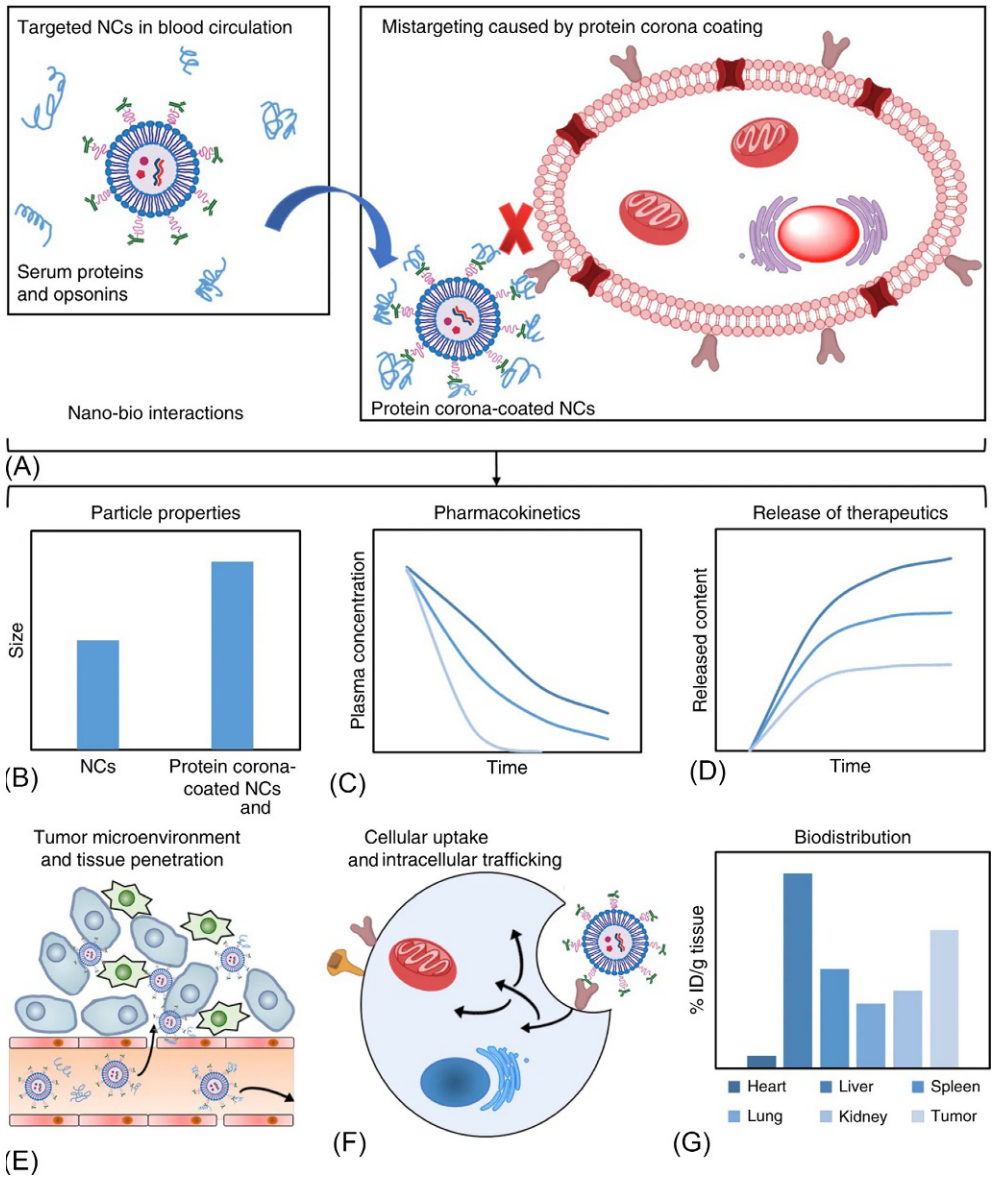

Fig. 8 The impact of nano-bio interactions on the systemically administrated NCs. (A) During systemic circulation, targeted NCs get coated with serum proteins and opsonins, which impacts the targeting efficiency and many other properties of NCs, including (B) particle size, (C) pharmacokinetics, (D) release profiles, (E) tissue penetration, (F) cellular uptake and intercellular trafficking, and (G) biodistribution (ID injected dose). *(Adapted with permission from D. Rosenblum, N. Joshi, W. Tao, et al., Progress and challenges toward targeted delivery of cancer therapeutics, Nat. Commun. 9 (2018) 1410).*

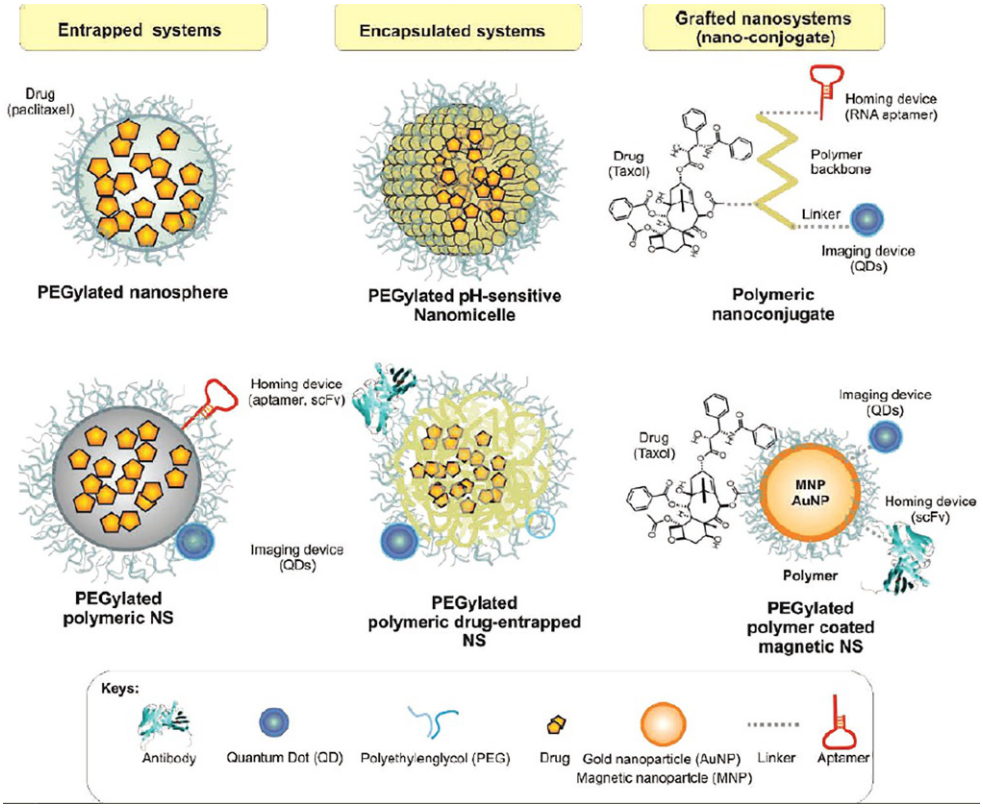

Fig. 9 Schematic illustration of nanomedicines with multifunctional adaptations. *(Adapted with permission from M. Fathi, et al., Int. J. Polym. Mater. Polym. Biomater. 64 (2015) 541–549).*

Taken all these understandings to the consideration, an ideal biodegradable polymeric drug delivery system for nonparental routs must be tailored in a way that it provides a number of imperative characteristics such as (a) suitable permeability and drug release profile based on physicochemical properties (e.g., lipophilicity and hydrophilicity) of cargo molecules, (b) biodegradability and biocompatibility, (c) tensile strength, and (d) possibility for surface modification and decoration.

3. Nonparental nanodrug delivery systems: Overview

Theranostic nanomedicines can be used for different purposes. By enabling a noninvasive assessment of the pharmacokinetics, the biodistribution and the target site localization of conjugated or entrapped pharmacologically active agents, nanotheranostics allow for the optimization of drug delivery systems. In addition, by combining information on overall target site localization with noninvasive imaging insights on the local distribution of the

drug and/or the carrier material at the target site, nanotheranostics can also be used for predicting treatment responses [49] Furthermore, by noninvasively imaging drug release in vivo, some of the basic properties of drug delivery systems can be visualized and analyzed, and attempts can be made to correlate the in vitro characteristics of carrier materials with their in vivo capabilities. Related to this, by using contrast agents to monitor the release of pharmacologically active agents from stimuli-sensitive nanomedicines, the efficacy of triggerable drug delivery systems can be optimized, as exemplified by several studies on thermosensitive liposomes. And finally, by providing real-time feedback on the efficacy of targeted therapeutic interventions, theranostic nanomedicines can also be used to facilitate (pre) clinical efficacy analysis, to prescreen patients, and to realize the potential of personalized medicine [50]. During phase I and phase II clinical trials, nanomedicine formulations could be labeled with radioactive compounds, in order to obtain some initial noninvasive information with regard to target site accumulation. On the basis of this, rational predictions could then be made with regard to the potential effectiveness of nanomedicine-based therapeutic interventions.

3.1 Oral nanodrug delivery systems

The site-specific delivery of the drug to the oral cavity can be used to treat a number of diseases of the mouth, such as stomatitis, periodontal disease, fungal and viral infections, and oral cavity cancers, thereby avoiding the first pass metabolism effect [51] (Table 1).

3.2 Colorectal nanodrug delivery systems

Colorectal-specific drug delivery systems are gaining importance for use in the treatment of chronic diseases, such as irritable bowel syndrome, inflammatory bowel disease, ulcerative colitis, and also for the systemic delivery of protein and peptide drugs. In the frame of colorectal cancer therapy, the most employed approach is the use of intravenously administered nanovectors in order to improve the pharmacokinetic behavior of otherwise problematic drugs [52]. The same concepts of pharmacokinetic improvement can also be applied to diagnostic nanovectors, in order to deliver a higher amount of labeling molecules to the site of colorectal cancers (CRCs), avoiding their toxic effects and improving their sensitivity. Despite the remarkable progresses in the development of more complex and efficient nanovectors, in the large majority of studies, the biological testing of nanoparticles still relies on 2D cell cultures and ectopic murine models of CRC. These preclinical models are well known and validated, but they give only limited insight into the potential clinical efficacy of the formulations in the study [53]. The 2D cell cultures are characterized by a simple and unrealistic environment in which cancer cell lines are forced to grow only on a surface. This condition can alter the cells gene expression and polarization, inducing a phenotype different from the one found in the actual CRC tissue. Use of these tissue-like environments and drug-loaded nanovectors provides

Table 1 Examples of commercially available nano-therapeutic products for oral administration.

Nanotechnology approach	Drug	Major indication	Drug Form	Brand name (manufacturer info)
Nanocrystals	Sirolimus	Graft rejection Kidney transplantation	Tablet	Rapamune (Pfizer Ireland Dublin
	Fenofibrate	Hypercholesterolemia	Tablet	Tricor/ Lipanthyl/Lipidil (Recipharm, Fontaine, FR)
	Aprepitant	Postoperative nausea and vomiting, Cancer	Capsule	Emend (Merck Sharp and Dohme Bv, Haarlem, NL)
Nanoemulsions	Cyclosporine	Prophylaxis of organ rejection following organ transplant	Capsules	Neoral (Novartis AG, Basel, CH)
	Ritonavir	HIV infections	Capsules	Norvir (Aesica Queenborough Ltd., UK)
Polymeric drugs	Sevelamer	Hyperphosphatemia Renal dialysis	Tablet	Renagel (Genzyme Ltd., Oxford UK)/Renvela (Genzyme Ireland)

Adapted with modifications from Hafner, et al., Int. J. Nanomedicine 9 (2014) 1005–1023.

unprecedented opportunities to study and exploit intercellular communication to achieve more specific targeting and even drug-free therapeutic actions (Fig. 10).

3.3 Nasal nanodrug delivery systems

Owing to nasal obstacles such as low membrane permeability, a short local residence time, and high turnover rate of secretion in nasal cavities, the bioavailability of nasally administered drugs is often comparatively poor [54]. The nasal drug delivery systems are promising adjuvant/delivery systems for nonparenteral delivery of antigens as well as for other immune-specific molecules. Moreover, the nasal administration of vaccines can induce specific IgA antibody responses at distant mucosal sites, including the upper and lower airway mucosa and the small and large intestines, as well as the nasopharynx, salivary glands, genital tract, and tonsils, because of the dissemination of antigen-specific

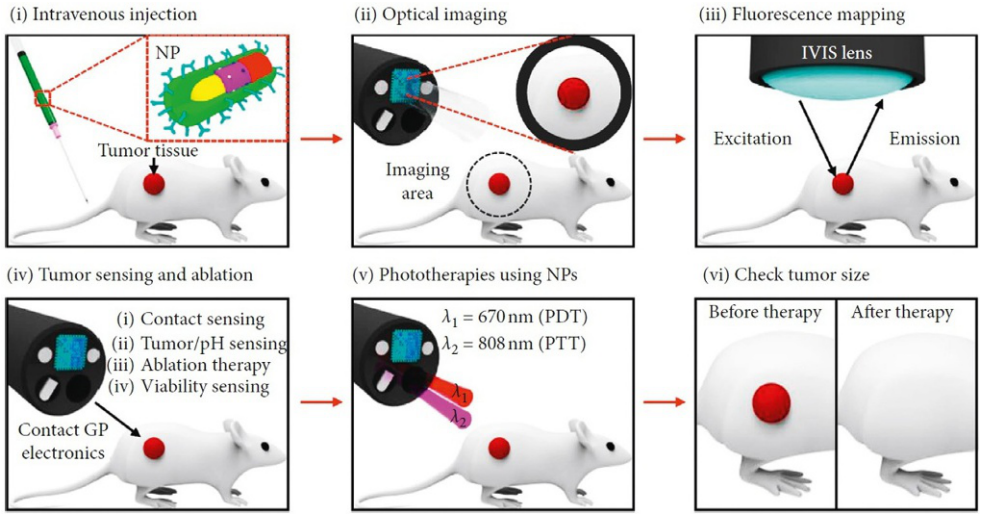

Fig. 10 Advanced nanotheranostic tools and strategies for endoscopic device development for the treatment of colorectal cancers. *(Adapted with permissions from R. Rampado, et al., J. Oncol. (2019) 740923. Copyright © Hindawi).*

lymphocytes in the common mucosal immune system [55]. The olfactory region is located at the top part of the nasal cavity under the cribriform plate in close proximity to the olfactory bulb, interlocking the nose with the brain. This region consists of three types of cells, namely the basal epithelial cells, sustentacular cells, and the olfactory neurons with their cilia extending toward the nasal cavity. After administration of the drug into the nasal cavity, the drug transport may occur through the olfactory epithelium, either (i) by axonal transport after internalization into the neurons, (ii) by paracellular transport across the spaces between cells and, notably across the channels next to the olfactory nerves, or (iii) by transcellular transport across the basal epithelial cells (Fig. 11).

3.4 Pulmonary nanodrug delivery system

Due to the complexity of respiratory disorders and lung morphology, it should be kept in mind that disease severity, age of the patient, breathing pattern, and device design, and structure decide the actual outcome of aerosol use and the final success of pulmonary therapy [56]. At the onset of the recent COVID-19 pandemic, enormous interest has been generated in the development of nonparenteral nanomedicine especially for treating Pulmonary Fibrosis. There was a recent development made in the formulation of a novel nanocarrier consisting of Lipoid S100 and chitosan or glycol-chitosan for the systemic delivery of low molecular weight heparin upon pulmonary administration. These nanosystems, formed by ionic gelation technique, provided both sufficient entrapment efficiency and mucoadhesive properties. Aerosolization of these formulations indicated

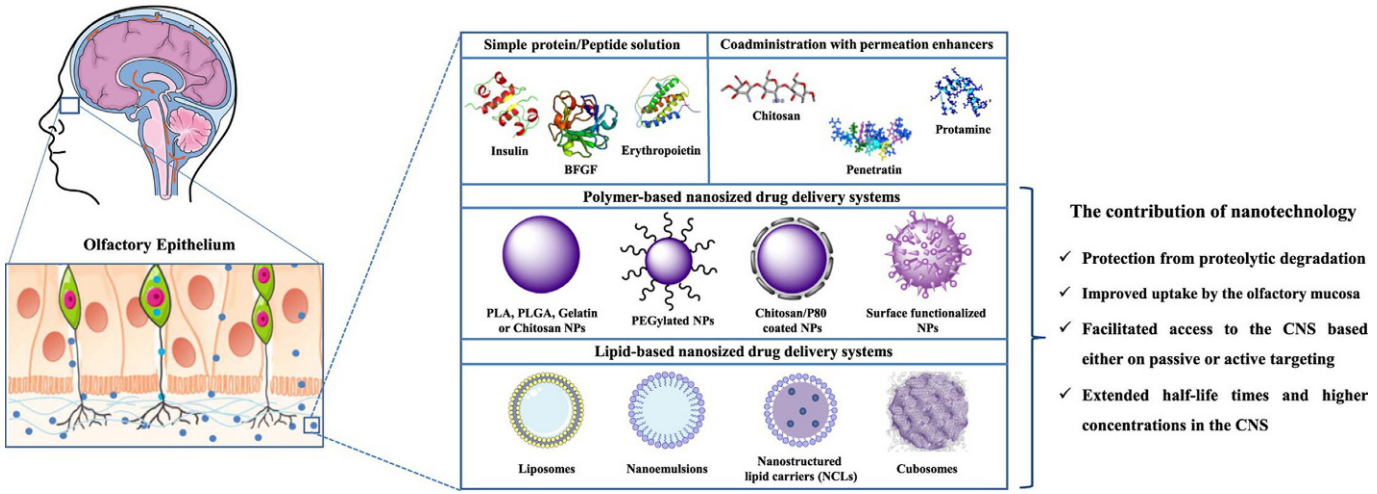

Fig. 11 Schematic representation of Nasal nanodrug delivery systems and possible uptake mechanisms involving olfactory pathway and transport of peptides from the nose directly to the brain. *(Adapted with permissions from E. Samaridou, M.J. Alonso, Bioorg. Med. Chem. 26 (2018) 2888–2905. Copyright © 2018 Elsevier).*

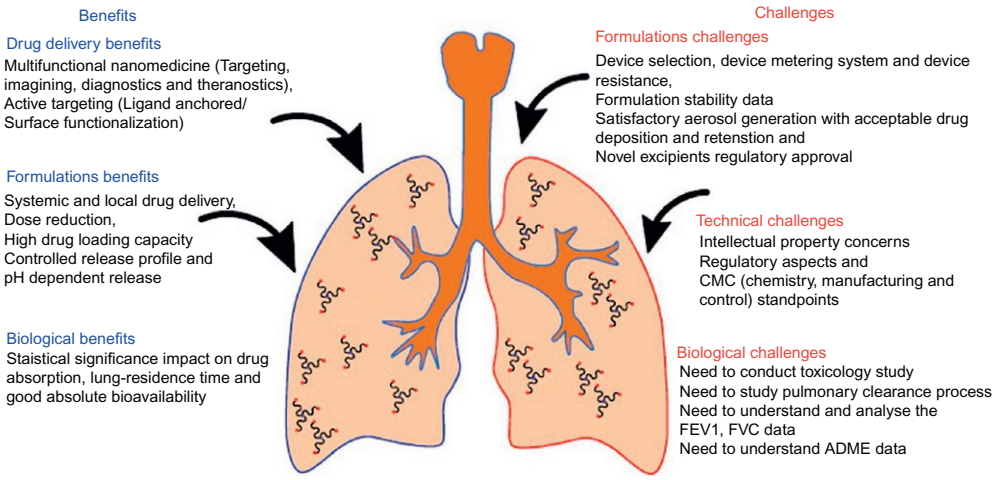

Fig. 12 Benefits and challenges of nanomedicines for pulmonary drug delivery. *(Adapted with permission from Mehta, et al., New J. Chem. 43 (2019) 8396. Copyright © 2019 Royal Society of Chemistry).*

that heparin could be delivered to the lung. Overall, these nanocarriers might have a use potential for systemic delivery of low molecular weight heparin as compared to the free drug with a therapeutic potential effect for the treatment of pulmonary embolism and other thrombo-embolic disorders [57]. Generally, the successful delivery of any active compound to the lungs by aerosol depends on four mutually dependent features: the formulation, the aerosol device design, the metering system and, finally, the patient's understanding/responsiveness (Fig. 12).

3.5 Transdermal nanodrug delivery systems

Transdermal drug delivery system refers to a route of drug delivery through the skin to achieve local or systemic therapeutic action. It is one of the focus areas of research for the third-generation pharmaceutical preparations, next only to oral medication and injection [58]. The reasons lie in the administration route of the drug, which is convenient, easy to use, noninvasive, and also improves patient compliance. It also reduces the fluctuation of the drug concentration in the blood, provides steady plasma levels and fewer chances of overdose and easy detection of the drug. At the same time, it evades the gastrointestinal environment, such as pH, enzymatic activity, and the interference of drug and food interaction on the drug efficacy and the "first pass effect" (where active drug molecules can be converted to inactive molecules or even to molecules responsible for side effects) by the liver [59]. Although TDDS has many advantages, the use of drugs in TDDS is currently limited. As mentioned above, the most resistance during the percutaneous permeation of the drugs comes from the SC of the skin. When many drugs are delivered through the

skin, adequate permeability rate is difficult to achieve as per therapeutic requirements. To overcome these difficulties, nanotechnology may be a good choice. Nanotechnology refers to the technology of using a single atom or molecule to produce or process macromolecular matter into a material with a particle size of 1–100 nm. One of the important areas of nanotechnology is nano-formulations [60]. Given their small particle size, nano-formulations have a better effect on drug retention, specificity and targeting, which makes an ideal TDDS. They have many advantages, such as being painless, minimal skin injury (does not change the general structure of SC of the skin and does not destroy the skin barrier function), and promotes permeation of macromolecular drugs, which has become a very popular field of research on TDDS. Nano-formulations can be divided into vesicles including liposomes, transfersomes, ethosomes, niosomes, invasomes, and nanoparticles including lipid nanoparticles, polymeric nanoparticles, and nano-emulsions [61]. As for active transdermal administration, microneedles are not involved, instead, ultrasonic, electroporation, hot perforation, and comprehensive application of other methods enhancing penetration are used.

3.6 Ocular nanodrug delivery systems

Anterior eye diseases are generally treated by eyedrops, but the rapid tear film turnover (15–30 s) will quickly dilute the eyedrops and drain the drugs through the nasolacrimal duct, and the remained drugs will have to penetrate the cornea to reach the anterior chamber [62]. Various ophthalmic vehicles, such as inserts, ointment, suspensions, and aqueous gels, have been developed in order to lengthen the resident time of instilled dose and enhance the ophthalmic bioavailability and for improving the retention and biodistribution of drugs applied topically onto the eye. The poor corneal penetration and retention of drugs, resulting in limited ocular bioavailability, require repeated instillations to achieve therapeutic drug concentrations in the eye. Topical eye drops are still the preferred dosage form because of convenience and good patient acceptance. The drug clearance typically occurs within 15–30 s owing to the tear film turnover, resulting in the intraocular bioavailability of topically applied [63]. One of the most investigated recent pharmaceutical forms is the in situ gels, which have been developed to prolong the precorneal resident time of the drug and to improve ocular bioavailability. The main challenge for retinal disease treatment is the ineffective drug delivery to the posterior segment. Owing to the nonspecific absorption and blood-retinal barrier, the systemic route delivers drugs to the eye at low rates with a high risk of systemic toxicity to other tissues. Many other factors also need to be addressed in detail, including the polymer purity; NP manufacturing technology, solvent residue, and potential local acidic environment during polymer degradation, material buildup in the eye after repeated dosing, foreign-body reactions, and the potential snow globe effects in the vitreous to disturb the visual axis. Success in the translation of nanomedicine would require a careful risk: benefit analysis, which is often skewed toward risk when it comes to novel therapeutics.

3.7 Regenerative nanomedicine systems

Nanotechnology applications to regenerative medicine have all the potential to revolutionize tissue regeneration and repair. However, the development of ideal nanomaterials capable of sending signals to the diseased or damaged cells and tissues to trigger the regeneration process still remains a challenge. In order to regenerate some loss or damaged tissue and organ, in vitro seeding and attachment of human cells onto a scaffold, followed by the culturing of the cells to form the new organ or tissue must be performed to avoid some transplantation of them. Scaffold design is a niche in regenerative medicine that involves creating a foundation for cell adherence that directs proliferation in an appropriate configuration and differentiation scheme. Nanoscale fibers have shown considerable success in the reparation and regeneration of soft tissues through tissue scaffolding in the skin, blood vessels, nerves, tendons, and cartilage applications [64]. Common design criteria include biocompatibility, porosity for cell growth and nutrient and waste flow, natural extracellular matrix (ECM) architecture, biodegradability at a rate consistent with new tissue growth, and mechanical support. One of the major applications in this field is the use of nanostructures having native tissue-mimicking ability, which has resulted in the development of long-lasting and better-performing scaffolds. Extensive research is being conducted on the use of scaffolds seeded with stem cells to generate bone and cartilage. However, the success of this technique is limited by the availability of stem cells and their efficiency in regeneration. The enhancement of axonal growth using nanofiber conduits for the treatment of neuronal injuries is also being explored [65]. Efforts are presently directed toward the development of nanofibers, which help provide properties similar to those of natural cardiac tissue. The clinical use of growth factors in wound healing has generated considerable research interest in recent years. Biodegradable scaffolds integrated with multiple growth factors appear to be the most promising therapeutic option for skin tissue regeneration. Progress made in molecular and stem cell biology, material sciences, and tissue engineering has enabled researchers to develop cutting-edge technology, which has led to the creation of nonmodular tissue constructs such as skin, bladders, vessels, and upper airways. In all cases, autologous cells were seeded on either artificial or natural supporting scaffolds. However, such constructs were implanted without reconstruction of the vascular supply, and the nutrients and oxygen were supplied by diffusion from adjacent tissues (Fig. 13).

4. Toxicity and safety concerns of nanomedicines

Regardless of various advantages, there are also some limitations associated with the usage of nanomedicine, particularly the possibility of generating toxicity at the cellular level. In this context, it is important to identify the properties to understand the mechanisms by which nanomedicines interact with living systems and to understand exposure, hazards, and their possible risks [66]. The toxicity of nanoparticles is currently a major issue in

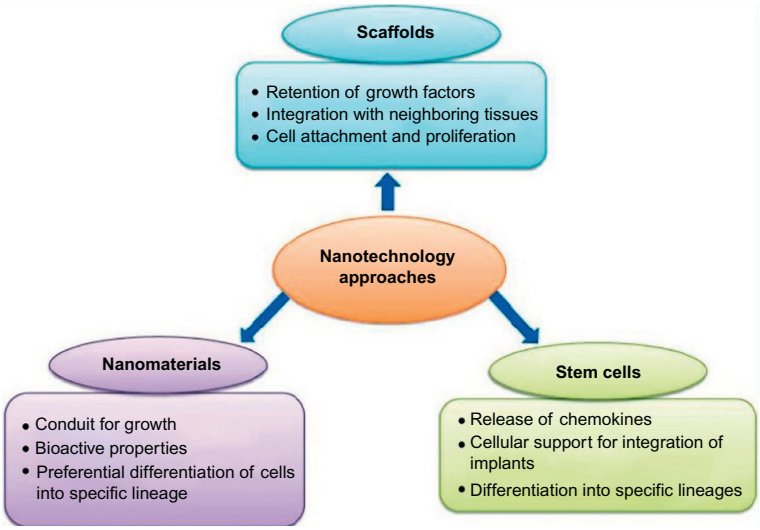

Fig. 13 Commonly used Nanotechnology approaches for regenerative medicine. *(Adapted from Chaudhury, et al., Int. J. Nanomedicine 9 (2014) 4153–4167).*

biomedical applicability since it is a multiparameter problem comprising of materials and morphological parameters such as composition, degradation, oxidation, size, shape, surface area, and structure. Nanomaterials are capable of disrupting the balance of the redox systems and, consequently, lead to the production of reactive species of oxygen (ROS). ROS comprise hydroxyl radicals, superoxide anion, and hydrogen peroxide. Under normal conditions, the cells produce these reactive species as a result of the metabolism [67]. When compared to micron-sized particles, nano-sized particles can be generally more toxic because they have a larger surface area (hence, more reactive), for a given mass, to interact with cell membranes and deliver toxicity. They are also retained for longer periods in the body (more circulation or larger clearance time) and, in principle, can be delivered deeper into the tissue due to their size. Hence, for understanding their pharmacokinetics it is important to define the critical parameters such as physicochemical properties, including size, size distribution, composition, surface characteristics, purity, and stability because they can directly affect in vivo activity of the nanomedicine. Nanomaterials must be evaluated for their toxic effects to assess their safety, along with the therapeutic agent itself. Examining how the nanomedicine and its components interact with blood and immune cells in vitro can help prevent serious and potentially lethal reactions during clinical evaluation. The immune response can directly rely on the adsorption pattern of body proteins. For example, during inflammation, certain matrix-degrading enzymes released by endothelial cells are adsorbed and migrate through the basal membrane and lead to angiogenesis; circulating nanomedicine targets this disturbed vasculature to eradicate the angiogenesis or stop its further spread across the endothelium to

access the joint cavity and other sites of inflammation. An assessment of the in vivo protein profile is therefore crucial to address these interactions and to establish biocompatibility. The clearance of nanoparticles is also size and surface-dependent. Small nanoparticles, below 20–30 nm, are rapidly cleared by renal excretion, while 200 nm or larger particles are more efficiently taken up by mononuclear phagocytic system (reticulo-endothelial system) located in the liver, spleen, and bone marrow.

4.1 Immunotoxicity of nanomedicines

Activation of the immune system is the most observed immune response in animal models, following the administration of nanomedicines. It is completely independent of the category of the nanomedicines. Moreover, excessive immune stimulation can result in autoimmune disorders and alternatively cause inflammation in tissues, resulting in long-term damage [68]. Nanoparticles can be taken up by immune cells, including monocytes, macrophages, platelets, dendritic cells in the bloodstream as well as within tissues such as Kupffer cells of the liver, dendritic cells in the lymph nodes and macrophages, and B cells in the spleen. Since the introduction of nanomedicines to the clinic, there have been several cases of acute immune responses to the NMP product in the form of hypersensitivity reactions, this is often due to the structural similarity of NM to viral antigens, which can trigger nonspecific humoral immunity and cause the complement system to produce an immediate eliminatory response. Endotoxin is a major contaminant in early nanomedicine formulations. If endotoxin levels are above certain thresholds, many immunotoxicity assays could give false-positive readings. Taking precautions early in the development process to reduce endotoxin contamination will allow for a more accurate assessment of the toxicity profile of the nanomedicine and its components. However, some nanomaterials can interfere with commonly used assays that assess contaminants and they may exaggerate the inflammatory properties of endotoxin. Controlling bacterial and endotoxin contamination is highly recommended before conducting toxicity or immunology assays (Table 2).

4.2 Challenges in the safety assessment

In spite of efforts to harmonize the procedures for safety evaluation, nanoscale materials are still mostly treated as conventional chemicals, thus lacking clear specific guidelines for establishing regulations and appropriate standard protocols. All nanoparticles rely on control at the nanoscale, meaning small variations may cause significant changes to the nanoformulation. However, not all techniques are sensitive enough to detect small changes in physicochemical properties, so orthogonal techniques are recommended for a more thorough evaluation. Despite the importance of surface evaluation, it remains one of the most challenging physicochemical tests. There are only a few widely applicable assays for surface characterization. Most assays must be individually tailored for the specific surface

Table 2 Major toxicity mechanisms identified during nanomedicine administration.

Types of toxicity	Trigger for toxicity	Consequences
Oxidative stress	Nanoparticle (reactive surface, dissolution of toxic ions); LMP; mitochondria dysfunctions; activation of immune cells	ROS toxicity; damage of other organelles; induce inflammation and geonotoxicity; apoptosis
Inflammation	Activation of TLRs and NLRs; uptake by immune cells; release of alarmins	NLRP3 inflammasome activation; release of cytokines
Genotoxicity	Nanoparticle interruption; ROS accumulation; Dissolution of toxic ions; inflammation	Chromosomal fragmentation, DNA strand breakages, point mutations, oxidative DNA adducts and alterations in gene expression profiles
Lysosome dysfunction (LMP)	Proton sponges hypothesis; ROS toxicity; Increase of lysosomal pH; Disruption of lysosomal trafficking	NLRP3 inflammasome activation; release of ROS, ions and hydrolytic enzymes; induce other organelles dysfunction; apoptosis
Mitochondria dysfunction	Mitochondria outer membrane depolarization; release of ROS	NLRP3 inflammasome activation; autophagy induction; apoptosis
ER stress	Unfolded protein accumulation of ER	Activation of ER stress signaling pathway and autophagy to balance homeostasis; apoptosis
Autophagy dysfunction	Blockage of autophagy reflex caused by particle overloading; excessive autophagy induction	Apoptotic and autophagic cell death

Adapted with modifications from Wang, et al., J. Mater. Chem. B 3 (2015) 7153–7172.

ligand-nanoparticle combination being evaluated. Among the most important limitations that can negatively impact the use of natural polymers as nanocarriers are their antigenicity and nonuniformity of properties from batch to batch. Variability in the composition is also accompanied by variability in trace impurities, cross-linking density, enzymatic degradation rate as compared with hydrolytic degradation [69]. The risk of viral infection in collagen and gelatin-based materials due to contamination with bovine spongiform encephalopathy is another drawback. Some of the advanced characterization techniques like reverse-phase high-performance liquid chromatography (RP-HPLC) and thermogravimetric analysis (TGA) can be used to quantitatively measure various surface coatings on a variety of nanoplatforms. Imaging by immunoelectron microscopy can also serve as a qualitative method to illustrate nanoparticle surfaces with the help of appropriate antibodies. Certain biological surface moieties have additional complexities that need to be elucidated through specific structural evaluations. For example, the specificity of targeting ligands can be assessed using immune-specific precipitation or titration assays

like ELISA, EIA, etc. Therefore, a combination of different surface characterization techniques along with biological assays may be required for molecularly targeted nanomedicines.

4.3 Strategies for engineering nontoxic nanomedicines

Combinatorial delivery of multiple therapeutic agents, not limited to chemotherapeutic agents, could potentially provide a strategy to combat drug resistance exhibited in many aggressive pathological cases. In addition to passively and actively targeted nanoparticles, targeting the intended disease site can also be achieved with stimuli-responsive drug delivery nanoparticles. New approaches have arisen from the pharmaceutical innovation and the concern about the quality and safety of new medicines by regulatory agencies. Quality-by-design (QbD), supported by process analytical technologies (PAT) is one of the pharmaceutical development approaches that were recognized for the systematic evaluation and control of nanomedicines. Responsive nanoparticles can be designed to deliver their cargo in reaction to some intrinsic or external stimulus. The payload can thus be released to the site of action upon the specific detection of stimulus and nanoparticles can thus undergo transition trafficking to the therapeutic site. Intrinsic stimuli can either be one or combinations of parameters like the pH, enzyme concentration, or temperature of the disease microenvironment [69]. Extrinsic stimuli consist of certain magnetic or electrical fields, ultrasound, or radiation. The goal of this dynamic design of particles is for improving drug accumulation at the site of action; however, assessing drug kinetics in this type of system requires additional understanding of the particle's mechanism of physical transition, the level of stimulation required, and drug release profiles before and after stimulation. Also, externally stimulated nanoparticles have the added complexity of potentially being a drug-device combination, which requires additional know-how and may complicate translation and adoption by physicians. In general, nanomedicines are designed to increase the half-life of the drug, enabling delivery of the active pharmaceutical ingredient (API) to its intended site of action. If the drug releases too quickly, it can produce off-target toxicities. On the other hand, if the formulation is too stable, the API will not be delivered in appropriate concentrations making it therapeutically ineffective. Drug release is, therefore, an important measure of nanoparticle stability. However, determining drug release in vivo is challenging because drug binding can equilibrate between the nanoparticle and abundant proteins in the blood.

5. Conclusion and future perspectives

Incorporation of nanomaterials for nonparenteral drug delivery application is an interdisciplinary research subject involving aspects of biology, medical science, material science, and nano-biotechnology innovations. The key focus of the subject is to achieve and reproduce multicomponent fabrication and designing that control and

measure property response at the nano-size scale efficacy. Biologists and Physicians should focus on ways to introduce multifunctionality without sparing enhanced performance and to increase biocompatibility and sustain enhanced multifunctionality in vivo. The first challenge stems from nanoparticle design and targeting with special emphasis to fine-tune the surface morphology, particle size, and surface charge determine pharmacokinetics, toxicity, and biodistribution. The efficiency of site-specific delivery depends on the profile of cargo-loaded MNPs, field strength, depth of target tissue, rate of blood flow, and vascular supply. Application-driven functionalization is a key ingredient for their successful multifunctional implementation in modern theranostics. Importantly, physicochemical properties of the nanoformulation need to be linked to their performance characteristics such as pharmacokinetics, biodistribution, efficacy, and toxicity profiles. Because of the demanding characterization needs, a clear advantage of the nanomedicine over existing formulations should be established early on in the development stage, along with a feasible manufacturing strategy to prevent expensive failures later on. Successful translation of research from academia to production lines has been identified as one of the major challenges in nanotherapeutic development. Strategies to foster and initiate this translation have yet to be developed to help European research institutions and industries remain competitive in global markets. A quick and successful translation of emerging nanotherapeutics is expected to adapt the established quality-by-design approach. The quality-by-design approach, in the field of nanotherapeutic development, promotes the idea that control over the quality, efficacy, and safety should be incorporated into the formulation development. This approach includes clear definitions of the desired performance (i.e., the expected specifications of the target formulation), nanoparticle design (i.e., the nanoparticle attributes providing efficacy and safety), manufacturing design (i.e., establishing the process parameters ensuring reproducibility of nanoparticle properties), and therapy design (i.e., the treatment modalities providing efficacy and safety of the therapeutic application). A process of developing an optimal formulation is influenced by a complicated matrix of interlinked or independent input and output parameters, which include critical process parameters, critical product quality attributes, and clinical properties such as safety and efficacy. For instance, in order to induce hyperthermia, a major objective is to control the heat distribution using multiple trajectories and also to enhance the formation of aggregates selectively on malignant cells. For magnetic resonance imaging, steps should be made for enhanced cellular internalization, slower clearance from tumor site and size-dependent tissue distribution. In the case of cell imaging and tracking, triggering should be improved to promote cell membrane receptor recognition, long-term in vivo monitoring, uptake initiation, and/or enhancement. Hence, multidisciplinary expertise and testing are essential to grasp a complete understanding of the design features that contribute to a safer and more effective therapy.

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CHAPTER 3

A regulatory framework for the development of topical nanomedicines

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1. Introduction

In the past few decades, the demand for better health-care solutions has boosted pharmaceutical companies to increasingly invest in R&D activities. Nanomedicines have been highlighted as one of the key enabling technologies, addressing both scientific and commercial interests [1, 2].

Due to their refined mechanical, chemical, metabolic, pharmacological, and immunological characteristics, nanoparticles are able to increase the therapeutic index of active substances through several mechanisms, including: (i) enhancement of delivery of molecules with poor physicochemical profile (e.g., low solubility and/or permeability); (ii) drug targeting; (iii) reduction of toxicological effects, by mitigating bioaccumulation processes; and (iv) establishment of triggered drug release, among others [3–6].

Nanomedicines biorelevant technological features make them a promising and strategic technology able to retain competitiveness and capitalize new markets [4, 7]. One of the most widespread clinical applications of nanotechnology concerns oncology area [8]. The encapsulation of a drug within a nanoparticle enables to modify its pharmacokinetic and pharmacodynamic properties, thereby enhancing the treatment efficacy and/or safety [8]. However, there is a need to define specific regulatory requirements in what concerns nanomedicine development, approval, and commercialization [4].

In the EMA reflection paper on nanotechnology-based medicinal products for human use, nanomedicines are defined as: "The application of nanotechnology in view of making a medical diagnosis or treating or preventing diseases. It exploits the improved and often novel physical, chemical and biological properties of materials at nanometer scale" [9].

Nevertheless, the ability of these nanosystems to alter pharmacokinetics (absorption, distribution, metabolism, and excretion) represents a source of significant scientific, toxicological, technological, and consequently, regulatory challenges [9].

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In fact, the regulatory agencies often suggest that nanomedicines should be regulated as "combination products," since it is not straightforward to establish a boundary that distinguishes them, from a "common" pharmaceutical, a medical device, or even a biological agent [7, 9]. Moreover, significant challenges regarding the evaluation of the quality, safety, and efficacy are also entailed during the development of nanomedicines. These are some of the underlying factors which explain that despite the considerable interest gathered by these technologies, the translation of nanomedicines to the market still continues to witness numerous hindrances [5, 10].

1.1 Academic gap

As of April 2019, PubMed returns 23,238 results to the search term nanomedicine, which reflects the academic interest on this subject. However, these numbers are significantly reduced when searching patents (2352) and clinical trials (353).

The identification of new therapeutic targets and the development of new drugs frequently relies on the literature, therefore, in our perspective, the reduced "bench to market" translation of nanomedicines can be ascribed to the early phases of technology conception, which are inextricably linked to academia [1, 11, 12].

Even though there are a plethora of highly promising proof-of-concept studies addressing nanoparticles, the evaluation of academics is essentially made through the number of publications, and not on successful technology transfer [10, 11]. As such, there is still insufficient concern being given to the industrial requirements of R&D activities, since the main emphasis is on innovation [11]. Often, the existence of robust characterization methods, the establishment of batch-to-batch reproducibility, or integrative understanding of particle biology interactions are not regularly addressed in a significant number of research papers [12–14]. The absence of such data explains the failure of a large proportion of nanomedicine findings, in what regards formulation reproducibility [12]. Moreover, when designing nanomedicines which require complex and/or laborious production procedures, scale-up problems are commonly not equated [3, 15], along with the toxicological profile of each component and respective metabolites [16].

Close collaboration between academia, industry, governmental institutions, and the regulatory agencies is thus essential to enable a successful transfer of methodological know-how during nanomedicines development [10]. Gladly, several initiatives have been addressing some of the abovementioned "scientific gaps." One interesting example concerns the overgrowing number of scientific publications on safe-by-design approaches to nanomedicines. These especially highlight nanoparticle toxicology and side effects, and have been a direct result of significant public research funding [10].

1.2 Toxicological restraints

Another important segment that conditions the approval of nanomedicines is biocompatibility, in other words "the ability of a material to perform with an appropriate

response in a specific application" [2]. In order to promote a proper safety evaluation of nanomaterials, it is imperative to have a sound knowledge on the system physicochemical characteristics [10]. Besides the properties inherent to the active substance, some of the most critical features behind toxicity and biodistribution are polydispersity, size, surface chemistry, and surface charge density [17]. It is essential to understand the influence of such properties on pharmacokinetics and pharmacodynamics, bioaccumulation, excretion, and toxicology mechanisms [7, 10]. It must also keep in mind that one of the gold standards behind nanotechnology applied to drug products concerns the enhanced permeability and retention (EPR) effect [12]. This effect, especially promising with anticancer drugs, reflects the ability of nanoparticles to accumulate in highly vascularized organs, such as the spleen or liver. Nevertheless, these occurrences (beneficial in some pathological scenarios) should be carefully monitored, since they can also be regarded as a limitation of nanosystems due to prolonged plasma half-life and higher area under the curve (AUC) levels [12]. For instance, when developing a topical nanomedicine, its "biopersistent" characteristics should be equated, since the penetration of nondegradable nanoparticles may compromise viable epidermis, and/or lead to accumulation in secondary organs following distribution [18]. In this context, nanomedicine-specific issues should be considered while developing a toxicological program [3]. For this, drug-nanosystem release rate and concentration on the action site, as well as off-sites, must be presented and compared with those including the free drug [3]. First, the evaluation of nanotoxicology is performed by in vitro studies, such as cytotoxicity assays. These provide some information regarding interaction mechanisms between the nanomaterial and the body. However, besides being rapid and cost effective, they fail to reproduce human body compensation mechanisms when exposed to toxics (biotransformation reactions) [2].

Current guidelines indicate that the same regulatory pathways and scientific considerations of conventional drug candidates are also applicable to nanomedicines. This calls for the use of toxicological testing in predictive rodents and nonrodent species [8, 19]. In vivo models do complement in vitro results, nevertheless, they present limitations as well [12]. During preclinical development of anticancer nanomedicines, the use of immunocompromised mice models, previously injected with cancer lines, is often performed. Nevertheless, when data are to be presented, not very often the immune status of the mice is taken into consideration, even though it could potentially play a role on tumor rate grow, biodistribution, clearance, and accumulation [12]. These "traditional" toxicological tests are time consuming, expensive, and more importantly the final results are not enough to establish a reliable comparison to what happens within the human body, thus hindering nanomedicines translation [2]. New assays that enable a more precise evaluation of nanomedicines are thus needed [3, 8, 12]. A useful approach is the in silico nanotechnology method, where toxicology is predicted based on a combination of computational and biostatistical methods [2]. Moreover, toxicogenomics can also provide significant insight on gene expression associated to nanomedicines intake [2].

The nanotoxicological classification system is also a valuable tool when designing a nanospecific toxicology program. This system enables grouping and targeting testing of nanomaterials, based on respective size and biodegradability, therefore, ensuring a predictive risk assessment [20–22]. For more details, the readers are suggested to go through an article written by Soares et al. [2].

1.3 Scale-up hurdles

As previously mentioned, there are a sheer number of parameters that exert a significant impact on nanosystems bioactivity (e.g., size, distribution, morphology, charge, purity, drug encapsulation efficiency, coating efficiency, and density of conjugated ligand/s) [3, 7, 23]. Therefore, it is crucial that these characteristics remain reproducible when produced at a larger scale. In this context, nanomedicines which do not require numerous manufacturing steps, such as lipid nanoparticles, can overcome some of the scalability complexities thus becoming more attractive from an industrial perspective [2, 3, 24]. Nevertheless, nanomedicines manufacturing frequently includes multiple steps, such as homogenization, sonication, milling, emulsification, and even for specific nanosystems, addition of organic solvents, proceeded by their evaporation [2]. Moreover, if the nanomedicine to be developed presents a complex structure (e.g., surface coatings and/or ligands, more than one drug encapsulated), batch-to-batch uniformity may be difficult to attain, thus compromising quality assurance and quality control, besides increasing production costs [3].

Under nonstandard manufacturing conditions, several factors may undermine the quality and safety of the final product: (i) incomplete purification of contaminants; (ii) insufficient batch-to-batch reproducibility, consistency, and storage, which may lead to diminished therapeutic efficacy; and also (iii) chemical instability of the encapsulated drug [3, 5, 6, 25].

1.4 Regulatory initiatives

As demonstrated in the previous sections, there are scientific, toxicological, and scale-up hurdles, which are conditioning the translation of nanomedicines to the market. However, in the past decade, the field of nanomedicine has matured considerably with several products in clinical trials, as well as some already commercially available [26].

Even though the lack of clear regulatory and safety requirements has affected nanomedicines development, nowadays, regulatory authorities have been directing efforts to overcome this situation [4, 26, 27].

Fig. 1 presents a schematic illustration of the main hurdles regarding nanomedicines translation.

A frequently mentioned regulatory-driven initiative concerns the nanotechnology characterization laboratory (NCL). This program aims to speed the development of nanomedicines for cancer patients, through the performance of extensive preclinical

Fig. 1 Hurdles to clinical translation of nanomedicines.

characterization of nanomaterials [11]. NCL has developed a robust framework for the sequential testing of toxicology, pharmacology, and safety properties of nanomaterials [16, 27]. Moreover, the FDA has issued four final and one draft guidances regarding nanotechnology in the FDA-regulated products. According to the draft guidance for industry on "Drug Products, Including Biological Products, that Contain Nanomaterials," issues such as the safety, effectiveness, public health impact, or regulatory status are to be discussed in a case-by-case process, for this reason, manufacturers are encouraged to take prior consultation with the agency in early phases of product development [8, 28].

Similarly, EMA due to the increasing demand from sponsors for scientific advice on nanomedicines has published in 2006 the first regulatory reflection paper on nanotechnology-based medicinal products for human use, and a webpage dedicated to the same topic [4, 9]. From then on, several nanomedicine-specific guidances have been released.

Table 1 summarizes the main guidances released by both the US-FDA and EMA on the nanomedicine field.

Furthermore, one of the most fruitful European initiatives in the nanomedicine field regards the European Technology Platform of Nanomedicines (ETPN). This association gathers more than 125 members from 25 different member states, and is specially focused

Table 1 Main guidances on nanomedicines.

US-FDA guidances
- Final Guidance for Industry—Considering Whether an FDA-Regulated Product Involves the Application of Nanotechnology (2014).
- Final Guidance for Industry—Safety of Nanomaterials in Cosmetic Products (2014).
- Final Guidance for Industry—Assessing the Effects of Significant Manufacturing Process Changes, Including Emerging Technologies, on the Safety and Regulatory Status of Food Ingredients and Food Contact Substances, Including Food Ingredients that are Color Additives (2014).
- Final Guidance for Industry—Use of Nanomaterials in Food for Animals (2015).
- Draft Guidance for Industry—Drug Products, Including Biological Products, that Contain Nanomaterials (2017).

EMA guidances
- Data requirements for intravenous iron-based nano-colloidal products developed with reference to an innovator medicinal product (2012).
- Data requirements for intravenous liposomal products developed with reference to an innovator liposomal product (2011).
- Development of block-copolymer-micelle medicinal products (2013).
- Surface coatings: general issues for consideration regarding parenteral administration of coated nanomedicine products (2013).

on regenerative medicine and biomaterials, nanotherapeutics, and medical devices [29]. The core research and innovation strategies regard the following diseases: atherosclerosis and other cardiovascular diseases, cancer, neurodegenerative/neurological disorders, infectious diseases, diabetes and endocrine disorders, and arthritis/osteoarticular pathologies. Moreover, nano-based solutions are also being explored for other medical sectors, including gastroenterology, dermatology, gynecology, and urology [4, 29].

These regulatory initiatives, supported by scientific, public health, and economic drivers, are a clear indication of multistakeholder efforts to accelerate translation of nanomedicines.

1.5 Nanomedicines in topical drug delivery

Cancer treatment is one of the main clinical applications of nanomedicines [30]. The increased complexity, combined with several specific nanomedicines action mechanisms (such as the EPR effect), are reasons that further justify this prevalence [31, 32]. However, as suggested by ETPN, other clinical applications are currently being explored. One of them relies on the dermatology area. Three main medical needs regarding this field can be pointed out: (i) development of reliable and competitive tools for early screening and diagnosis; (ii) noninvasive treatment of melanoma and nonmelanoma

cancers; and (iii) regeneration of the skin, grafts for management of chronic wounds and ulcers induced by aging, vascular disorders, and rare diseases [29].

Even though a plethora of elegant therapeutic options can be equated to address these medical needs, topical drug products offer a noninvasive and painless route for drug administration, which reinforces patient compliance [33–36]. There are, however, important physiological barriers that impair skin drug delivery.

Four different layers can be identified in the skin: the outermost—*stratum corneum* (SC), viable epidermis, dermis, and subcutaneous tissue. The first layer is composed by corneocytes, proteinaceous cellular compartments interconnected by desmosomes. These elongated and nucleus absent cells are embedded within an interstitial lipid pathway [37]. Due to its highly keratinized structure embedded within lipid bilayers, SC has a high density and low hydration being, for this reason, the main barrier for percutaneous absorption [38]. Dermal absorption across the epidermis can occur via three pathways: the intercellular, transcellular, and the follicular route (less expressive). Since these are not mutually exclusive, dermal absorption commonly occurs through a pathway combination. In spite of these routes, topical drug administration is challenging because SC is practically impermeable to foreign molecules. Moreover, after topical application, drug losses can occur via desquamation, sebum secretion, components evaporation, and even by clothing scrub [33, 34, 39, 40].

Even though some drugs present high efficacy toward cutaneous diseases, their usefulness is often compromised by poor skin permeation, linked to the abovementioned biological barriers [41].

From a technological and therapeutic point of view, nanoparticles present several physicochemical characteristics that enable them to enhance drug permeation and consequently, augment its local action: (i) small particle size; (ii) high specific surface area; (iii) high drug loading; (iv) drug protection from degradation; (v) possibility of deformation (e.g., transferosomes); and (vi) biocompatibility/biodegradability (e.g., lipid nanoparticles), in particular solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) share a chemical similarity toward skin lipids. Moreover, their solid nature favors the formation of a film that leads to an occlusive effect that prompt drug penetration [18, 41, 42].

These reasons further justify the interest in developing nanosystems especially tailored to deliver therapeutic agents to the *stratum corneum*, deeper skin layers or even systemically [18]. Some examples of topical nanomedicines market approved or under clinical development are listed in Table 2.

2. Pharmaceutical development of a topical nanomedicine

There are several literature reports that identify and explore the main hurdles associated with nanomedicine translation. However, in this review, the key objective is to establish

Table 2 Examples of topical nanomedicines in clinical development stages and market approved.

Active substance	Nanomedicine class	Pharmaceutical dosage form	Therapeutic indication	Status	Ref.
Amphotericin B	Liposomes	Gel	Treatment of cutaneous leishmaniasis of *Leishmania* species *major* and *tropica*.	Phase II	[43]
Nanosilver	Metal-based nanosystems	Cream	Infection diseases (fungal and bacterial)	Phase I	[44]
Nanosilver	Metal-based nanosystems	Gel	Infection diseases (reduce potential pathogen microbial loads in mechanical ventilation patients)	–	[45]
Nanosilver (SilvaSorb)	Metal-based nanosystems	Gel	Wound dressing	Market approved	[46]
Capsaicin	Nanoparticles	Cream	Diabetic neuropathy	Phase 2 and Phase 3	[47]
Paclitaxel	Nanoparticles	Ointment	Cutaneous metastases from nonmelanoma cancer in adults	Phase 1 and Phase 2	[48]
Paclitaxel	Uncoated nanoparticles	Ointment	Psoriasis	Phase 1	[49]
Diclofenac sodium (Oxalgin NanoGel)	Nanoemulsion	Gel	Analgesic and antiinflammatory	Market approved	[50]
Tretinoin (Nioret nano gel)	Nanogel	Gel	Acne vulgaris	Market approved	[51]
Thiocolchicoside (Zyflex Nano gel)	Nanogel	Gel	Joint and muscular pain	Market approved	[51]
Tetracaine (Optisome)	Lipossomes	Gel	Local anesthetic	Market approved	[51]

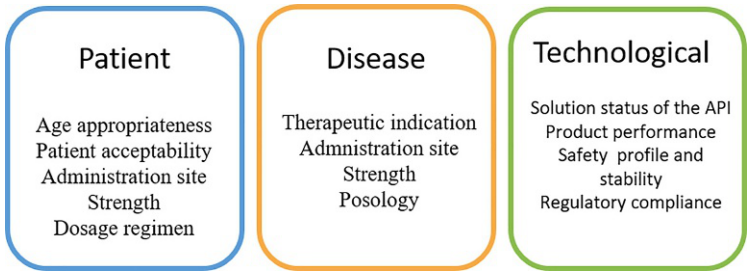

Fig. 2 Main regulatory requirements which should be addressed while defining the quality target product profile of a topical nanomedicine [52]. Key: *API*, active pharmaceutical ingredient.

a framework for the pharmaceutical development of topical semisolid nanomedicines, based on the regulatory requirements of topical dosage forms.

The pharmaceutical development process must be based on a sound scientific basis and present a clear narrative of each step involved in product development. First, the quality target product profile (QTPP) should be clearly defined. When defining the QTPP, it is of outmost importance to balance patient, disease, and technological aspects. The following scheme presents some of the main issues within each area (Fig. 2).

Based on these assumptions, the pharmaceutical dosage form should be selected. The three most common semisolid dosage forms for topical products are ointments, creams, and gels [34].

2.1 Drug and nanosystem selection

One of the primer considerations should be a careful assessment of the physicochemical and biological properties of the drug to be delivered.

Even though nanoencapsulation can overcome solubility issues, special attention should be paid to the log P, pKa, molecular weight, stability, solubility, as they are useful parameters to predict diffusivity and/or partitioning across the *stratum corneum* [34, 53]. Moreover, sensitivity to light, air, or moisture, degradation pathway, and polymorphism should be also carefully assessed.

Based on the drug properties and primary therapeutic target, the type of nanosystem should be selected. Two major classes of nanosystems can be considered: (i) lipid based and (ii) polymeric nanocarriers. Lipid systems include micelles, liposomes, microemulsions, nanoemulsions, solid lipid nanoparticles (SLNs), and nanostructured lipid carriers (NLCs). Among them, liposomes are the oldest nanotherapeutic platform, being already clinically approved [25, 53, 54]. These nanovesicular systems are composed by amphipathic phospholipids arranged in one or more concentric bilayers, which enclose an aqueous core.

Even though several methods have been reported for the production of these vesicular systems, the most common is film hydration [23]. In this technique, a lipid film

constituted by superimposed lipid bilayers (e.g., soy lecithin:cholesterol, phosphatidylcholine:cholesterol) is obtained after rotary evaporation of an organic solvent or cosolvent, wherein the lipids were previously dissolved [55]. Afterwards, the lipid film is hydrated with an aqueous solution, thus forming liposomes. As previously mentioned, to avoid scale-up issues, nanoparticle production methods should be as simple as possible. In this context, there could be potential issues arising from this method. Taking this into account, the solvent injection method appears to be a more reliable strategy to obtain higher batch-to-batch reproducibility. Accordingly, phospholipids and other lipophilic substances are dissolved in ethanol. Afterwards, this phase is rapidly injected into a large amount of aqueous buffer, resulting spontaneously in vesicle formation. Through this method, liposomes are prepared under mild conditions and in one single step, thus potentiating a simpler scale-up process [55].

There are, however, other production methods with high scale-up potential, such as the high-pressure homogenization, or the membrane contact method, which are commonly employed in the production of lipid nanoparticles (SLN and NLC). For further details on the specificities of the available nanosystems and corresponding production methods, the readers are suggested to go through an article written by Paliwal [6, 24, 56, 57].

2.2 Excipients

Simplicity is the basis of good formulation design and the shorter the ingredient list, the better [37, 58]. Nevertheless, a complex combination of excipients is often essential to meet patient requirements, as well as to achieve dermal drug delivery. For these reasons, excipients in topical drug products account for more than 90% of the formulation.

During their selection, from a regulatory perspective, it is common practice to choose excipients with extensive published safety data and already listed in international pharmacopeias [34, 59]. Moreover, during excipient selection for topical drug products, their intrinsic batch and source variability (e.g., homolog composition of hydrocarbon chains, the degree of unsaturation, molecular weight, and polymorphism) should be taken into account. These factors are directly linked to product variability, which may have a direct repercussion on the product rheological profile, microstructure/physical properties, crystallization of the active substance or other ingredient, stability, or bioavailability [52].

Therefore, factors such as the source and production method of the selected excipients should be carefully screened and accounted for [60]. Moreover, the following aspects should be mentioned: (i) excipient function; (ii) batch and source variation; (iii) quantity of each excipient; (iv) excipient grade and respective critical quality attributes; and even (v) when excipients are presented as a mixture of compounds, detailed qualitative, quantitative, and rheological data. In addition, if a permeation enhancer is to be used, detailed information on their action mechanism should be provided, as it impacts drug permeation and, therefore, bioavailability [52].

2.3 Formulation development and optimization

Formulation development is based on the previously defined QTPP. Bearing in mind the ideal features of the product (e.g., organoleptic characteristics, performance attributes, and therapeutic indication, among others), the critical quality attributes (CQA) of the formulation can be identified. Afterwards, based on the prior knowledge, a risk assessment analysis is made. In this stage, it is intended to gather up systematically all the possible variables that could influence or generate a quality failure [23]. These variables may be further classified into two groups: (i) critical material attributes (CMA) and (ii) critical process parameters (CPP).

Preformulation studies should be performed to optimize the nanosystem and its incorporation into the pharmaceutical semisolid dosage form. The main focus of these preliminary tests is to screen solubility and to assess the compatibility between the components of the formulation [34]. Based on this knowledge, a design of experiments approach should be followed in order to identify the optimal settings of both CMA and CPP. The application of this approach (also known as quality by design) during the pharmaceutical development process enables a greater understanding of the product and its manufacturing process. By doing so, it is possible to establish a design space, which can then create the basis for a more flexible regulatory assessment [61]. This rationale is essential to document the reasons which supported the selection of the nanosystems and the excipients.

2.3.1 Technical challenges

Once selected the dosage form, type of nanosystem, drug, and excipients, it is essential to provide further information on several factors. According to the draft guideline on quality and equivalence of topical products, there are specific regulatory requirements that should be presented. The following sections present an adapted summary of these requirements aiming the development of a topical nanomedicine.

Formulation microstructure characterization

An adequate characterization of the system is essential to obtain reliable data with high translation potential [62]. Moreover, as the microstructure impacts both safety and efficacy of the product, the regulatory authorities require a careful description of this parameter.

Regarding nanoparticle microstructure characterization, the particle size, polydispersity index, and zeta potential represent crucial features of the formulation, being frequently considered as critical quality attributes. Even though these parameters can be easily obtained by dynamic light scattering (DLS), the FDA in the draft "Guidance for Industry on Drug Products, Including Biological Products, that Contain Nanomaterials" recommends the use of complementary methods when measuring critical material attributes [28]. These can include: nanoparticle tracking analysis, atomic force microscopy, particle size determination by sedimentation, and laser diffraction [62].

It is important to further discuss the effect of scale-up, excipient interactions, and batch variation in the product microstructure. As previously mentioned, difficulties during scale-up are one of the main limitations of nanomedicines translation, since the microstructure characteristics of nanoparticles are greatly influenced by the manufacturing processes. Moreover, product microstructure and physical properties are often complex in semisolid products [52]. In this context, a careful assessment of the final product microstructure is required.

Active substance form and saturation degree in the drug product

Nanoparticle production yields a colloidal system, where the drug, according to its physicochemical characteristics and affinity degree for the lipophilic phase, is solubilized in the lipophilic or in the hydrophilic component. Factors such as the partition of the drug between the lipophilic and aqueous phases and its solubility bear a significant impact on skin permeation [59, 63]. In this context, information regarding drug encapsulation efficiency (EE), as well as the drug loading (DL) within the nanoparticle system should be provided. Furthermore, since semisolid dosage forms are generally required for skin delivery, it is important to determine the specific concentration of drug in each phase (lipophilic/hydrophilic) in the final product [52].

Formulation components with the ability to modulate the drug permeation profile should also be described (e.g., permeation enhancers and retarders), since the concentration gradient of the active substance between the drug product and the site of action will change with the addition of such components [64]. Their action mechanism should be discussed.

Risks of precipitation, particle growth, and change in crystal habit

The stability of the encapsulated drug in the final dosage form should be carefully assessed.

Lipid components, present in the colloidal system, are prone to undergo polymorphic changes arising from storage or temperature variations. These polymorphic changes can modify the nanoparticle crystalline structure, leading to an "expulsion" of the entrapped drug. This occurrence alters the dermal bioavailability [59]. In this context, the presentation of the nanoparticle-drug thermal stability is required. This can be achieved through differential scanning calorimetry (DSC) experiments. Moreover, accelerated stability studies also represent a valuable source of information. It should be denoted that DSC experiments can also provide a valuable input in what concerns the evaluation of the drug solubility status within the nanosystem. If the drugs are solubilized, their melting transition peaks should be absent in the thermograms [57]. In nanostructured lipid carriers, DSC also enables the calculation of the drug crystallinity index. If the crystallinity of the drug-nanoparticle sample is lower when compared to the bulk solid lipid, it means that there is an increased number of lattice defects in the nanoparticles.

The disorganization of the lipid matrix promotes a higher loading capacity and encapsulation efficiency of the drug [65–67].

In addition to thermal stability, information regarding the stability of the formulation on short-term, real-time, and accelerated conditions should be presented [34]. Analytical centrifugation allows the rapid measurement of a separation process, through the analysis of transmission profiles and the respective instability index values. Higher transmission profiles can be linked to different instability phenomena, such as gravitational separation (creaming/sedimentation), flocculation, coalescence, Ostwald ripening, or phase inversion [68].

Patient acceptability and usability

Consumer or patient should always be at the forefront whenever developing a topical medicine [34]. It is expected that a formulation to be topically applied presents acceptable organoleptic and usability characteristics in order to reinforce patient compliance. In this context, the assessment of the product's texture profile, through a texturometer, should be performed. This test enables the determination of the spreading properties, such as hardness and compressibility, as well as other characteristics, including adhesion of the formulation to the skin (adhesiveness); elasticity, which describes the rate at which the deformed sample returns to its original condition after the removal of the deforming force, and finally, cohesiveness, a parameter that provides information on the structural reformation following formulation application [69–71].

Transformation of the topical product on administration

Another regulatory requirement, which can be considered a challenge, concerns the evaluation of the product transformation on administration, a concept identified in the literature as "product metamorphosis" [52].

Upon product application, most of the dermatological vehicles undergo considerable changes. These are mainly induced by mechanical shear associated to product application, or to solvent evaporation. As a result, the initial structural matrix of the system is altered, which may then lead to unwanted drug crystallization phenomenon [72–74]. Several reports have shown drug crystallization as a possible cause of poor drug permeation in the skin. Therefore, a careful assessment of the interactions among the excipients, the drug and the skin is required by the regulatory authorities [52]. A paper by Goh et al. presented a rapid and simple approach using ATR-FTIR spectroscopy to identify drug crystallization in the skin for simple formulations of sodium diclofenac. The authors were able to apply multivariate data analysis to visualize and differentiate drug crystals in superficial layers of the *stratum corneum* [73]. In order to assess drug crystal formation in deeper layers of the skin, the same research group successfully used localized nanothermal analysis and photothermal microspectroscopy as surrogate methodologies [74].

Performance attributes

Nanotechnology offers several advantages in topical applications, however, quantitative studies able to establish a relation between nanoparticle dose and exposure, with skin penetration and therapeutic efficacy are still lacking [56]. Nevertheless, there are "standard" product performance tests, regulatory accepted, which can provide further information on this topic: in vitro release testing (IVRT), in vitro permeation testing (IVPT), and tape stripping.

a. In vitro release testing

The release profile of a topical semisolid dosage form, acquired through IVRT, enables the determination of the in vitro release rate (IVRR), a kinetic parameter which provides important information on the microstructure characteristics of the product, such as particle size, and rheological behavior [75, 76]. As the active pharmaceutical ingredient must be released before it can diffuse and become bioavailable in the skin, the determination of the IVRR is considered a CQA of the product [52, 77–79]. One important aspect which should be taken into account is that IVRT setup includes an artificial membrane, which does not resemble the skin's *stratum corneum*. When selecting a membrane, there are several features which need to be considered: (i) the membrane should properly hold the formulation, but it cannot be a barrier for the active substance. A porous structure with minimal thickness is, therefore, highly desirable [80, 81]; (ii) no physical or chemical interaction should exist between the membrane and the formulation and (iii) it is also of the outmost importance that the membrane does not release any leachables that could interfere with the assay [81, 82].

An interesting paper by Kaur et at. evaluated the permeation of a novel amphotherin-B nanogel and nanoethogel. The authors concluded that the prepared nanosystems presented enhanced skin permeation and deposition effect in several biological and artificial membranes. Moreover, the authors demonstrated similarity of Strat-M, an artificial membrane, toward human skin, documenting IVRT as a suitable quality control for topical drug performance [41].

For the development of IVRT methods, several guidelines and reference studies should be considered: draft guidance on quality and equivalence of topical products [52], the guideline on quality of transdermal patches [83], the draft guidance on acyclovir [79], and also the work by Tiffner et al. [78]. Moreover, validation studies of IVRT should also be performed, and should include: IVRT laboratory qualification studies, IVRT method validation studies, in which membrane inertness, linearity, precision, sensitivity, discriminatory power, and robustness must be determined.

b. In vitro permeation testing

The permeation profiles obtained from IVPT can be used to assess topical pharmacokinetics, providing for this reason one of the most useful and insightful in vitro information [75, 84].

The usage of biological membranes, especially human, is required since it closely mimics in vivo conditions [85]. There are, however, important features that should be considered when developing a discriminative IVPT method:

- Design specific methods for blinding and randomization according to ICH E8, to minimize risks of bias.
- Establish precise criteria in what regards the selection and preparation of the skin. Factors such as anatomical region, condition, and duration of skin storage should be considered. Skin with tattoos, scars, or with significant follicular density should be excluded. Furthermore, evidence should be provided to demonstrate that the preparation technique and storage does not introduce artifacts, nor alters the skin barrier function [52]. Skin integrity should be evaluated prior and after each permeation experiment.
- Skin from different donors should be used, at least 12 with at least, two replicates per donor [52].

c. Tape stripping

The dermatopharmacokinetic method, commonly referred as tape stripping (TS), is a minimally invasive in vivo procedure in which tape strips are sequentially applied and removed from the skin surface [34]. Through this technique, *stratum corneum* layers are collected in each tape strip, being their content processed by suitable analytical methods [86, 87]. The principal assumption of this procedure is that the amount of drug collected in the strips throughout time, represents the rate and extent of the drug penetration in the skin. In other words, this method enables the determination of the drug dermatopharmacokinetic profile. In the EMA draft guideline, specific operational requirements of TS are presented. These are based on the "two-time" approach, developed by Professor Richard H. Guy and Professor Annete Bunge. This experimental method considers (i) solely one uptake and one clearance time; (ii) strict cleaning procedures that assure that the formulation excess is properly removed prior to tape stripping; (iii) removal of nearly all *stratum corneum* during the experiment (and, as such, most, if not all of the drug); and finally, (iv) inclusion of the first tape strips. EMA requires that a minimum of 12 subjects must be considered [52].

2.3.2 Product characterization

Once the lead candidates of the formulation are defined, it is essential to provide a detailed characterization of the product. This information will define a provisional drug product specification list [34].

General guidances on the drug product specifications are given in ICH Q6A, Q3B, Q3C, and Q3D and the European Pharmacopeia dosage form monographs. Characterization data should include a representative number of batches (not less than three), since

disperse systems usually exhibit variation. Table 3 presents the specification parameters of topical semisolid dosage forms.

The following flow chart intends to present the key events of a nanomedicine topical semisolid formulation development program (Fig. 3).

Table 3 Semisolid drug product specifications parameters.

Parameter	General considerations
Organoleptic characteristics	Product visual appearance may include color changes, absence of aggregates, and/or formulation appearance. Odor is also an important parameter, since there are components which are prone to hydrolysis or microbial contamination (e.g., triglycerides). The product should also be characterized with microphotography [52].
Pharmaceutical dosage form	The solution state of the active substance, the disperse and immiscible phases and dosage form type should be presented (e.g., active substance in solution in the oily phase, two phase vehicle: o/w cream).
pH	The pH can influence the solubility and stability of a drug, the preservatives effectiveness and also the viscosity of the drug product. However, when designing a product for topical application it must be considered the skin's normal pH (5–6). If a product with different pH is used, the potential to cause skin irritation needs to be evaluated, taking into account the administration site and dosage regimen.
Assay	The specifications for drug content usually range between 95% and 105%.
Content uniformity	Formulation uniformity is especially relevant in biphasic systems. It is usually determined by the analysis of the drug content from sampling of the top, middle, and bottom of a bulk sample or alternatively, using process analytical technologies to provide a real-time analysis.
Related substances, degradation products and residual solvents	Impurities, degradation products, residual solvents are considered CQA of the drug product, due to their potential to affect safety and efficacy. Limits for maximum daily dose of degradation products should be properly estimated and presented. This may be a challenging task, since treatment duration and posology can be highly variable in topical drug products. Moreover, specific precautions in calculating acceptance limits for impurities should be made for cutaneous products applied to damaged skin or products containing penetration enhancers [52]. The ICH guideline on impurities in new drug products should be followed [88].

Table 3 Semisolid drug product specifications parameters—cont'd

Parameter	General considerations
Microbiological attributes	Microbiological aspects should also be a part of the product specification. The need for a microbial quality test is highly dependent on the dosage form. Typically, it is used for nonsterile products with high water content or whenever an excipient susceptible to microbial contamination is used. Pharmacopeia microbial limits should be followed. If the product is intended for deep wounds, severely damaged skin, administration prior invasive procedures, or for preparations for irrigation, its sterility is mandatory. In such cases, the sterilization method should be considered during preformulation studies and sterility testing should be performed in the final packaging, in order to assess container closure integrity [52].
Preservative content	For nonsterile products in multiple-use containers, the need to include a preservative should be addressed and justified. The concentration should be the lowest possible. For multiphase formulations, the preservative solubility in the different phases should be assessed. In addition, preservative effectiveness should be evaluated during the ongoing product stability program. To comply with all regulatory requirements, the European Pharmacopeia 5.1.3., Efficacy of antimicrobial preservation test should be followed.
Rheology and viscosity	Rheological properties should be detailed for semisolid formulations, as changes in these properties can be indicative of fluctuations in the product stability or performance (e.g., viscosity decrease in gels can be related with possible breakdown in molecular weight of the polymer due to microbial contamination) [34]. Semisolid dosage forms usually display non-Newtonian flow behavior, therefore a complete rheological analysis encompassing the following parameters should be presented: • Complete flow curve of shear stress *vs* shear rate. Multiple data points across the range of increasing and decreasing shear rates should be included, in order to identify any linear portions of the upcurves or downcurves. • Yield stress and creep testing; • The linear viscoelastic response (storage and loss modulus *vs* frequency); Rheograms should be provided and the product's behavior classified according to shear and time effects (e.g., pseudoplastic, dilatant, thixotropic). As the rheological profile is susceptible to scale-up effects, it is essential to verify these properties during product development [52].

Continued

Table 3 Semisolid drug product specifications parameters—cont'd

Parameter	General considerations
Performance attributes	Appropriate tests to characterize product performance, such as in vitro release, in vitro permeation and tape stripping should be developed and support formulation stability during storage [52].
Container closure system and package integrity	The container closure system selection should be discussed and justified. Aspects such as the choice of materials, protection from moisture, oxygen and light, drug product compatibility, dosing, usability, and safety should be carefully considered. Sterile drug products should be packaged in single-use containers. If any device is co-packaged to facilitate administration, it should be CE-marked and the compatibility between the device and the product, should be addressed. Moreover, if this device is intended for measuring or application of the product, the dose reproducibility and accuracy should be demonstrated [52]. The suitability of the measurement device shall comply with the test "Uniformity of mass of the released doses" according to monograph <0672> Liquid preparations for oral use of European Pharmacopeia. General principles on packaging materials used for human drugs and biologics can be found in Guidance for Industry, Container Closure Systems for Packaging Human Drugs and Biologics.
Stability program	Once characterized, the formulations are placed under stability long term, and accelerated conditions. The designated shelf life is then established based on the product characteristics throughout storage. These should comprise physical, chemical, microbiological, rheological and performance characteristics. Requirements for special storage conditions should be addressed (e.g., do not refrigerate) [52].

3. Conclusions

Nanomedicines claim several advantages regarding conventional drug products (e.g., increased drug bioavailability, safety, targeted delivery, and occlusive effect). These can be of great interest when considering a topical application. There are, however, several constraints which have been delaying nanomedicine translation to the market: (i) scale-up hurdles, which limit the industry interest in these systems; (ii) lack of scientific knowledge on nanotoxicological characterization methods; and (iii) the absence of clear regulatory requirements. In spite of these scenarios, there are several initiatives supported by scientific, health, and economic drivers, which are working collectively to develop more robust platforms, able to stimulate nanomedicine translation. Reasons which

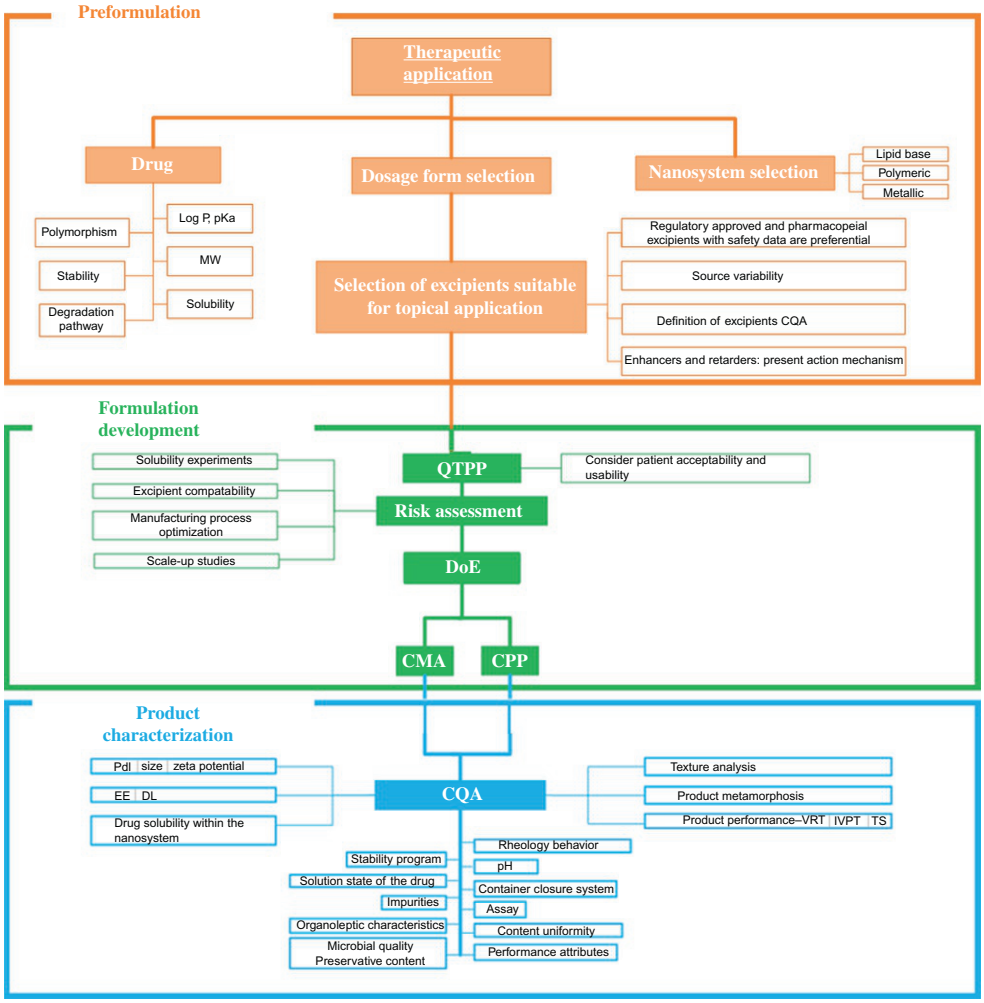

Fig. 3 Key events of a nanomedicine topical semisolid formulation development program. Key: *CMA*, critical material attributes; *CPP*, critical process parameters; *CQA*, critical quality attributes; *DL*, drug loading; *DoE*, design of experiments; *EE*, encapsulation efficiency; *IVPT*, in vitro permeation testing; *IVRT*, in vitro release testing; *PdI*, polydispersity index; *QTPP*, quality target product profile; *TS*, tape stripping.

reinforce these initiatives are (i) the increased clarity on the therapeutic value of nanomedicines; (ii) the possibility to promote the industrial pharmaceutical development based on QbD; (iii) multiple efforts regarding a more inclusive assessment of nanotoxicology; and finally, (iv) the release of several nanomedicine-specific guidelines.

The development of a topical nanomedicine is unique to a particular drug, nanosystem, and dosage form. The recently issued EMA draft guideline on quality and

equivalence of topical products, even though specially conceived for topical generic products, can provide a basis for a patient-driven pharmaceutical development process, easily adapted for a topical nanomedicine. Despite the several challenges concerning microstructure characterization, active substance presentation, patient acceptability, product metamorphosis, and performance attributes, this guidance in conjunction with the current European regulatory framework can provision a general overview of the development program.

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CHAPTER 4

Physiology of the biological barriers

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1. Introduction

Site-specific delivery of therapeutic agents is an utmost necessity to improve the efficacy of the therapy and to reduce the associated side effects. Delivery of the therapeutics to the site of action is a great challenge, as the disease site is not exposed to the therapies; several biological barriers prevent the formulation to deliver the therapeutic agent to the site of action. Among these barriers, cellular membrane [1], blood-brain-barrier [2], blood-vessel fenestration [3], gastrointestinal barrier [4], cancer microenvironment, phagocytic system, and multidrug resistance in cancer cells [5] are limiting the passage of therapeutics to the desired sites. The basic mechanism of these barriers to prevent any invasion of foreign particles (e.g., xenogens and pathogens) within the system to protect our body from them and simultaneously establish homeostasis for physiological functions. These mechanisms similarly limit the therapeutic agents to reach to the particular disease sites of the body. Circumvention of these barriers is of utmost important characteristics to deliver the agents to the desired area, accordingly, formulation scientists are focusing on delivering the drugs using different novel approaches or developing special devices to overcome the associated problems [5–7]. Along with circumvention of these barriers, these novel formulation approaches could help the therapeutics to deliver at targeted sites to avoid systemic exposure of the therapeutic agents, and thereby reduce associated side effects [7, 8]. This chapter will include different biological barriers that impede the delivery of therapeutics as a normal physiological role of protecting the system.

2. Biological barriers

Based on the previous illustrations, it is clear that the pharmacological activity of the therapeutic agents predominantly depends on the stability of the formulation and targetability in the biological system [9, 10]. Thus, illustration of the major biological barriers would be furnished in this section.

Theory and Applications of Nonparenteral Nanomedicines
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2.1 Gastrointestinal barrier

The gastrointestinal tract of the human system is one of the largest mucosal epithelia forms an important barrier between the internal and external environment. This barrier composed of glycoproteins mainly secreted from the goblet cells protects the health due to its prime role in the regulation of the immune system [4, 11]. The mucosa of this system is an aqueous layer acting as a semipermeable membrane to perform its multifaceted task, which allows passage of nutrients and immune sensing, while this 100–150-μm thick membrane limits the transportation of injurious components, such as microorganisms and antigens [12]. Thereby, this mucosa acts as a filter for the larger molecules, with a weight greater than 600 Da [13]. Following successful penetration of the agents through this mucosal layer, it reaches to the epithelial cells of enterocytes for further penetration within the systemic circulation. In order to create such a specific barrier, the orientation of the structural components and their molecular interactions retain the integrity of the barrier and immune homeostasis. Thus, if there is any damage to the mucosal barrier in the intestinal tract, the function of the barrier is also compromised, and thereby there will be an invasion of external components within the system [14].

The single columnar epithelial cell layer under the mucus are forming a tight barrier for the penetrated materials from the mucous layer, because of the presence of tight junctions, thus the penetration of orally administered drugs are also hindered [15, 16]. Different cells are present in these layers, which include goblet cells, enterocytes, paneth cells, and endocrine cells. The number of goblet cells is found to increase from the small intestine (10%) to the distal colon (24%) [13]. There are four different regions are available in the gastric epithelium based on the structural configuration, which includes the pyloric region, the glandular fundic region, the cardiac region, and the nonglandular stratified squamous, where every region has its own function to serve in the gastrointestinal system. For example, the cardiac region controls mucus and bicarbonate production, whereas the fundic region secretes hydrochloric acid and pepsinogen and the region of stratified squamous allows it to resist food abrasion [17]. The epithelial cell layers are lined with villi and crypts, where the surface of is of the intestine is increased greatly by the presence of these microvilli for the absorption of nutrients to the systemic circulation, alternatively, crypts take part in the renewal of the damaged cells [16, 18]. The apical side of the epithelial layer is exposed to the lumen where the basolateral side is attached to the gastrointestinal tract lumen. Further, the epithelial layer is supported by the presence of connective lamina propria [19]. This lamina propria composed of several components within it, such as blood vessels, lymph vessels, nerve cells, and smooth muscle cells. This section acts as the bridge between the food compartment region that is the lumen side and systemic passage of the nutrients.

There are four different mechanisms are involved for transportation of drugs or other foreign materials to transport from the apical side to the basolateral side, i.e., to systemic

circulation, which includes passive diffusion of molecules from one side to the other side, transcellular transport, paracellular transport, and carrier-mediated transport and vesicle-mediated transcytosis. The mechanism of transport of the molecules from one side to the other is largely depending on the molecular weight and size of the compound, its ability to act to the plasma membrane of the cells, physicochemical properties including partition coefficient of the compound, and stability and surface charge of the compound. For example, the hydrophobic nature of the drug will help to partition the drug to the cell membranes and follows the transcellular pathway to cross the barrier (Fig. 1). Thus, if a drug component having optimized balanced on solubility in aqueous and lipid environment, could be able to cross the barrier via the transcellular pathway [20]. Alternatively, the lipidic barrier could not be crossed by the hydrophilic components, where these components adopt a passive diffusion pathway to transport the drugs from the higher concentration region to the lower concentration region. Additionally, these molecules can also choose the paracellular pathway to transport, however, limited to molecules with a smaller size (<11 Å) [21]. On the other hand, tight junctions at the apical side of the cells limit the passage of larger molecules from this area, further the presence of adherens junctions and desmosomes creates strict cell adhesion bonds to support the barrier integrity [4, 22]. These tight junctions are connecting two adjacent cells on their membrane, which are created by the protein-protein interaction. Mostly, the proteins and peptides follow

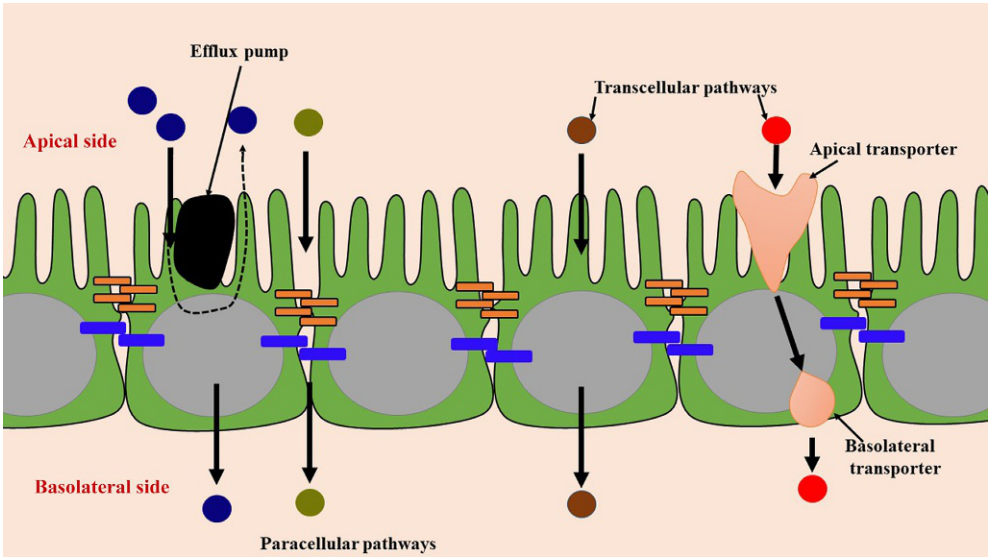

Fig. 1 Different absorption and transportation modes of drugs including the barriers of transportation from the cells of the intestinal epithelium: efflux pump, paracellular transport, transcellular transport, and carrier-mediated transport (may be vesicle-mediated transport).

the paracellular routes of drug transport, where larger proteins do not permeate because of their size and presence of tight junction [22, 23]. An alternative pathway to transport drug includes receptor-mediated endocytic pathway, where the drug molecule binds to the specific receptor on the cell surface, followed by internalization of the complex to deliver the drug into the cell. Here the ligand, i.e., the drug molecule would be recognized by the receptor of the cell membrane on the apical side of the lumen, thus, peptide transporters on the cell membranes found to improve the bioavailability of certain drugs conjugated to specific amino acids [24, 25]. Finally, the presence of P-glycoprotein (P-gp), an ATP-binding cassette (ABC) transporters, alters the intracellular distribution of drugs and efflux out the drug from the cells to the luminal side [26, 27]. Certain specific features of the molecule are recognized by the efflux pump that expels the absorbed drug from the membrane of the epithelial cell [26, 27].

2.2 Cellular membrane

It is obvious to get the internal and external compartments of fluid separated in different organs of a vertebrate animal, and thus predetermination of the penetration characteristics of the formulation is highly important to fulfill the desired intention.

In the case of skin, the outermost layer, the epidermis comes in contact with the environment, where the outermost membrane of the epidermis, i.e., stratum corneum acts as the rigid barriers to provide protection. Apart from the corneocytes on the stratum corneum, the other layers of the epidermis are composed of different layers of keratinocytes with different characteristics. These multilayers are known to form stratified epithelium of this biological barrier, where the tight junctions connect the keratinocytes. There are different expressions of tight junctional proteins observed at different layers of the epidermis [28].

The cellular membrane of the kidney creates the barrier along the renal tubule that separates filtrate from the glomerulus, i.e., the urine, and renal parenchyma. The presence of junctional proteins of different characteristics on the epithelial cells at different segment of nephron influences the paracellular transport of components in the urine. For example, claudin-1 is a small transmembrane protein, usually found in distal collecting segments of the tubule abundantly, which is responsible for sustaining the barrier function; whereas claudin-2, the channel forming tight junction proteins creates leaky epithelia, usually found in the proximal segment of renal tubule resulting in higher paracellular permeability of small cations and water molecules [29, 30]. Expression of these junction proteins differ in a different diseased condition, thereby the absorption of different therapeutic agents differ in renal disease conditions [31].

Similarly, the blood-biliary barrier and bile ducts are maintained by the barrier at the localized area along the bile canaliculi. Localization of tight proteins is also reflected in this case, basically found to be expressed within the apical region of the hepatocytes that

forms the epithelial cells of the bile duct and bile canaliculi. The abundant presence of tight junctional proteins in the liver revealed its important role in the maintenance of bile secretion, paracellular permeability, and cell polarity [1]. These tight junction proteins are also involved in facilitating viral (hepatitis C) infection, whereas the change in tight junction protein expressions has been reported in liver cancer cases [32].

The cellular barrier of the lungs creates an active barricade at the airspace and blood interface, where the expression of more than 25 types of different claudins create chief cellular essentials of the barrier. Among the claudins, claudin-18, claudin-4, and claudin-3 are the most noticeable junctional proteins where alteration of the junctional proteins weakens the barrier function. This weakening is mostly observed in several diseased conditions, leading to the formation of pulmonary edema [33, 34].

Similarly, we have a rigid biological barrier in the intestine, in testes, and in the brain. The blood-testes barrier is located between the neighboring Sertoli cells of the seminiferous tubule. Discussion on the intestinal cellular membrane has been made in the previous section, whereas the rigid blood-brain-barrier of the central nervous system would be discussed in the next section.

2.3 Blood-brain barrier

The protection of the central nervous system from the external environment is furnished by the presence of the blood-brain barrier. The blood-brain barrier describes the distinctive characteristics of the microvasculature of the central nervous system. These continuous nonfenestrated barriers are found in the endothelial cells of cerebral capillaries and of choroid plexus. This barrier regulates the transportation of cells, ions, and other molecules tightly from the circulatory blood to the central nervous system [35, 36]. The endothelial cells of the blood-brain barrier restrict heavily to critically maintain neuronal function via protecting the central nervous system from disease mediators, injury, inflammation, pathogens, and toxins. The presence of the restrictive barrier creates a major obstacle for the therapeutics, thus major efforts are furnished to deliver drugs to the central nervous system through different formulation approaches [37]. Several neurological disease states, such as neurodegenerative disorders, brain traumas, multiple sclerosis, and stroke alter the rigidity of this biological barrier, thereby increasing the permeability of foreign materials to the central nervous system [35, 36].

The early concept on blood-brain barrier toward its rigidity revealed that the presence of endothelial cells in the blood capillaries in the brain is responsible for the rigid barrier characteristics [38]. Later in 1987, the influence of astrocytes on the rigidity of this biological barrier was explored and they added that the presence of astrocytes in invertebrates augments on true barrier properties of the endothelial cells, however, they do not add in vertebrates [39]. Two means of such rigidity in the blood-brain barrier have been demonstrated by the ultrastructural studies, which differentiates the endothelial cells in

the brain and peripheral capillaries. The presence of limited number endocytotic vesicles limits the transcellular transportation of components from the circulation, where the other is due to the presence of tight junctions in the endothelial cells, thereby limiting the paracellular transportation as well. The pericytes are usually found around the brain capillaries, where their innervation is not continuous, partially cover the endothelial cells (Fig. 2). The combination of these two mechanisms protects the central nervous system from invading microorganisms and chemicals. A distinct perivascular extracellular matrix, a layer of basal lamina 1 encloses the endothelial cells and pericytes. This extracellular matrix differs in composition to basal lamina 2, which encloses the glial endfeet bounding the brain parenchyma. Projections from the neuronal axons onto the arteriolar smooth muscle are responsible for the regulation of cerebral blood flow through the production of vasoactive neurotransmitters and peptides. The peptides and other agents produced within the cells may also be responsible to regulate the permeability of the endothelial cells. Microglia are the resident immunocompetent cells of the brain [41].

Therefore, this rigid barrier in blood capillaries allows us to cross the small lipophilic molecules, concurrently completely inhibits permeation of other molecules. Smaller or even larger hydrophilic molecules are usually followed by an active transportation pathway to cross this rigid barrier [42]. Other than that, a high concentration of some specific membrane transporters on the epithelial membranes on brain capillaries are constantly present to transport essential nutrients from the circulatory blood, such as glucose and

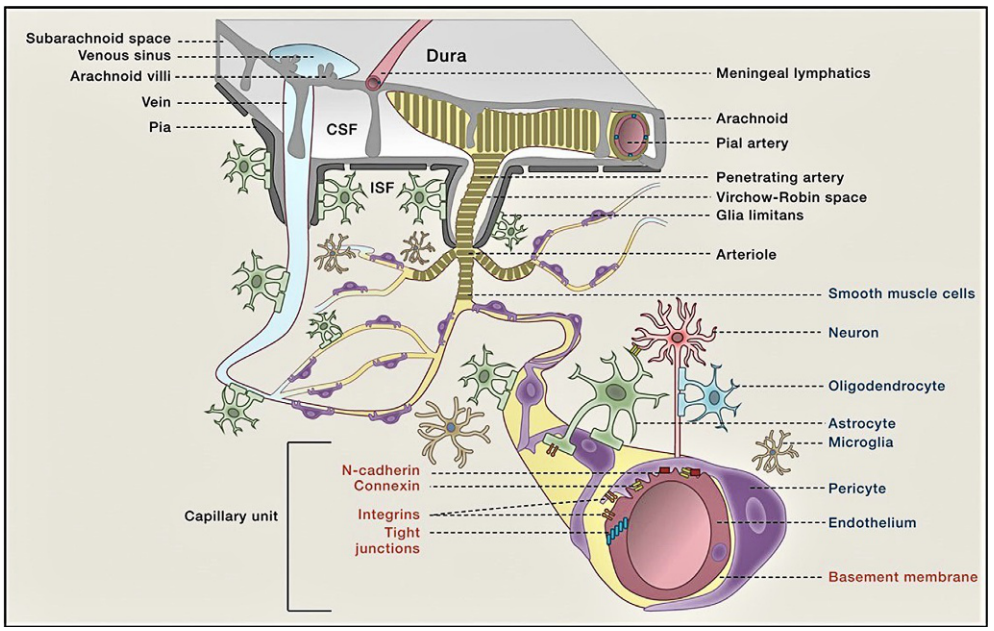

Fig. 2 The cell arrangements at the blood-brain barrier [40].

certain amino acids or allied molecules. Alternatively, the presence of the number of specific receptors for certain macromolecules including physiologically relevant peptides, which when binds to that particular molecule allows passage of the molecule into the brain. Few examples of such receptors include glucagon-like peptide 1, insulin, angiotensin II, interleukin-1, and transferrin. Study outcomes of previous researches revealed that transportation of specific respective agonists is allowed to enter into the brain [43]. Contrarily, transportation of cytokines and other growth factors are limited to be transported across the blood-brain barrier [44]. Similar to the gastrointestinal barrier, P-gp efflux transporters are abundantly available at the brain capillaries, and their role is to efflux back the absorbed drugs to the circulation. That is exactly the opposite phenomenon of drug absorption where the lipophilic molecules, which entered into the epithelial cells or brain cells, are transported back to the circulation. Knockout animals to this P-gp protein have shown to improve the permeability of P-gp substrate molecules while targeting therapeutics to the brain [45], at the same time P-gp inhibitors are also found to block the activities of P-gp transporters at the epithelial cell, thereby preventing the efflux of the absorbed drug. Subsequently, the concentration of drug at the target site increases [46].

As mentioned above, the tight junctions of the endothelial cells of the central nervous system are highly responsible for creating resistance against paracellular transportation of the ions and molecules. It has been revealed that the homotypic and heterotypic interaction between cellular molecules of the endothelial cells creates this tight junction through the formation of cellular adhesion on the apical side of the capillary membrane and brain. Depending on the selectivity of each region to permeate unionized molecules (<4 nm), the composition and strength of the barrier varies [47]. The above discussion suggests that the molecules larger than 4 nm could penetrate the blood-brain barrier through the discontinuities in the junctions.

This junction of the transmembrane cells contains different molecules, including occludins, claudins, and JAMs, where these claudins are tetraspanins available in more than 25 categories. Evidence are available to support the role of claudins in the formation of a paracellular barrier [48]. Depending on the amino acid residues in the extracellular loop of the claudins reflect the charge and size selectivity of the cellular pore, thus compositions of the claudins play an important role in the determination of barrier properties of the junction [49]. As discussed earlier, different claudins are responsible to form resistance to different molecules at different regions of the body, thus the expression of these claudins differs from area to area. The research revealed that the expression of claudin-5 is higher at the central nervous system. Also the evidence of the presence of claudin-12 and claudin-13 in the blood-brain barrier is available to support the rigidity of the barrier [50, 51].

Occludin expressed on the epithelial cells of the central nervous system is also tetraspanin, which is responsible to create this tight junction to the brain [52]. Compared to

claudins, expressions of occludin is much higher in the central nervous system, however, functional and the high-resistance blood-brain barrier is observed in the animal models with deficient occludin [53]. Alternatively, the JAMs are members of the immunoglobulin superfamily, which usually responsible to form homotypic interaction at the tight junction of the blood-brain-barrier. The presence of JAMs has been revealed to regulate paracellular permeability including leukocyte extravasation [54]. Among the superfamily members, Daneman and the team reported the presence of JAM4 in the blood-brain barrier of mice [51].

2.4 Infrastructure of the blood vessels

Mechanical properties of blood vessel structure play an important role in several disease condition. Typically, the length of the blood vessels increased to 40%, and simultaneously the circumference is increased to 30% when a person is in normal blood pressure. Usually, there are three different layers found in the blood vessels, the tunica intima, i.e., the inner layer; the tunica media, i.e., the intermediate layer; and the tunica adventitia, i.e., the outer layer. The outer and the middle layer of the blood vessels are very much developed in the large arteries, which constitute most of the tissues [55].

The presence of blood supply is essential for the survival of the surrounding cells, as this circulation provides oxygen and nutrition to the cells, at the same time the metabolic products are excreted from the site. Cancer cells are not an alternative; these cells are also in need of nutrients and oxygen, which they usually obtain from the surrounding blood vessels. However, at the later stage of cancer cell proliferation, the need of growth components, which are essential for rapid propagation, is increased. This need for excess nutrients leads to the stage to create more blood vessels to the affected area, to promote angiogenesis [56]. This increased blood supply aids in fulfilling the high demand of growth components, such as fibroblast growth factor (FGF), platelet-derived growth factors (PDGF), and vascular endothelial growth factor (VEGF), and thus the level of these growth factors are found to be increased within the cancer cells [57]. Simultaneously, overexpression of FGF receptors (FGFR), PDGF receptors (PDGFR), and VEGF receptors (VEGFR) receptors on the vascular smooth muscle and endothelial cells in the tumor microenvironment allows increased entrapment of the components to that area and stimulate to promote angiogenesis of tumor microvessels [57]. In due course of the process, the presence of growth factors eventually acts on subendothelial layer of the blood vessel and increases the leakage or porosity of the cancerous microenvironment. Increased porosity affects the permeability of the area, leading to the enhancement of macromolecule transportation [58]. This enhanced porosity, and thus permeability facilitates enhanced permeability and retention (EPR) of the circulatory macromolecules, provides a novel approach to target the cancerous microenvironment via entrapment of therapeutics within a compatible nanocarrier [26]. However, abnormal angiogenesis

is the consequence of the uneven expression of these growth factors to the cancer site, which may further influence the EPR. Further exploration of this mechanism has opened up several research options including the development of antiangiogenic therapies through targeting these proangiogenic targets, which are in process to treat and cure cancer [59]. Further, the application of VEGF to the human colon tumor xenograft animal models has shown to enhance the porosity of the blood vessels at the cancer site, to allow the macromolecules more that leaky area [60].

Targeting to improve permeability thus directly related to increase EPR effect at the tumor microenvironment and therefore to improve the porosity of the affected area several artificial means have been explored. Apart from the application of growth factors, as discussed earlier, researchers are focusing on increasing fluidity via increasing the temperature of the affected area, or by application of tumor necrosis factors. The local increase in temperature of the affected area has shown to alter the extracellular matrix of the applied area, thereby magnetic nanoparticles or light activation hyperthermia had shown to increase the therapeutic potential of the chemotherapies through the improvement of permeability by affecting collagen organization [61].

2.5 Phagocytic system

The concept of phagocytic cells was first demonstrated by the activity performed by Ribbert during the early 20th century. The research included peripheral injection of lithium carmine solution where the researcher demonstrated the presence of a group of cells, which are responsible to capture and clear them from the circulation. Thus, the cells are being stained because of uptaking the injected molecules [62]. Subsequent analysis of those cells demonstrated to be mononuclear phagocytosing cells, which were named differently by different researchers, 'histiocytes,' 'polyblasts,' 'clasmatocytes,' etc. Subsequent research on this topic through the incorporation of different dyes, the fibroblasts, and phagocytes was differentiated, and Karl Albert Ludwig Aschoff has described this group of cells as 'reticuloendothelial cell' (RES) [63]. Two terms, 'reticulo' and 'endothelial' are introduced to represent the inner meaning of the system where 'reticulo' refers to the tendency of these particular large phagocytic cells toward various organs and 'endothelial' states its similarity to the vascular endothelium [62]. The major function of this RES system was described to capture and clear the unwanted foreign or damaged internal particulate matters in the circulatory system.

Various novel drug delivery systems are formulated in the nanometric size range to improve their EPR effect in the circulation. Such the EPR effect of those formulations allowed the formulation to be delivered to the site of action during their stay in circulation. Further, it has been described that various blood proteins, such as albumin, globulin, and fibrinogen and other proteins, alter the fate of these nanocarriers in the circulation [64]. Usually, following the administration of the nanocarriers into the

circulatory system, these circulatory proteins interact with the nanocarrier structures and get absorbed onto the surface of the nanocarriers [5]. Adsorption phenomenon of the proteins on the nanocarrier surface forms the nimbus, called 'proteins corona.' The quantity and the composition of proteins on the corona are varied in different carriers because of different interaction between the plasma proteins and nanocarriers, and also protein and protein [65]. Best on the nature of the corona, they are divided into two categories: soft corona, and hard corona. A higher affinity of the circulator proteins toward the nanocarrier forms the hard corona, whereas the lower affinity of the proteins toward the nanocarrier gives rise of the soft corona. However, the quantity of protein(s) on the surface of the nanocarrier along with its arrangement onto the nanocarrier surface solely depends on biological interaction between the proteins and nanocarrier and on the physicochemical condition [66]. Uncontrolled and unplanned formation of proteins corona leads to an effect on in vivo stay of the nanocarrier, which possibly may lead to a change in allocation, targeting, and opsonization characteristics of the nanocarrier and may also lead to produce unintentional toxicity of the formulation. However, this adsorption phenomenon of proteins on the carrier surface can be planned for the known protein to favor drug delivery system, especially in targeted delivery systems of therapeutics [65].

2.6 Cancer microenvironment

Incurability and relapse of different cancers are the common reasons for drug resistance in the cancer microenvironment. There are several factors in the tumor microenvironment that are responsible for the creation of such a barrier to the delivery of therapeutics to reach the cancerous area. If the therapeutic agent crosses the vascular endothelial barrier, it may reach the cancerous environment. This microenvironment of the cancerous area may widely vary in different cancer, location, and stage of progression [67]. The cancerous microenvironment creates an unfavorable environment to the delivery system, thereby prevent penetration of the therapeutics into the cancer cells to encounter an essential therapeutic response. The cancer microenvironment comprises of extracellular matrix (ECM) that hampers the therapeutics to cross the microenvironment. This ECM consists of proteoglycans and matricellular proteins, the cross-linked structure of fibrillar collagen, and hyaluronic acid to reach the target site in cancer cells. This ECM provides the structural integrity of the cancer microenvironment, where the ECM allows the passage of oxygen and nutrients to the cancer cells to regulate progression and tumorigenesis [68]. As discussed earlier, the complexity of the ECM barriers differs in different cancers, where the use of collagenase found to disrupt the ECM barrier to assist diffusivity of the formulations to the site of action. The presence of this ECM barrier has shown to restrict therapeutics to enter into the cancer microenvironment, thus these therapeutic agents are not allowed to reach the target cells to result in desired efficacy [66, 69].

Following sufficient growth of the cancer cells in a particular region, to proliferate more and to obtain sufficient growth factors and nutrients, the cancer cells dislodge from its origin, enter into the circulator system, and result in metastasis. The cells travel to multiple areas of the body, colonize to those areas, and start growing individually away from its original place of origin. Several proteolytic enzymes are responsible for such dislodging of the cancer cells from its origin and to confiscate the barrier for the metastasis process [70]. The proteolysis process of the ECM is facilitated by the presence of a family of a proteolytic enzyme, matrix metalloproteinases (MMP). Thus, the level of this enzyme in the circulatory system may be acting as a biomarker in cancer conditions to diagnose the stage of progress [71, 72].

Uncontrolled proliferation of the cancerous cells moves the cells away from its origin and from the vasculature, thereby the concentration of oxygen was found to decrease proportionately as the distance increases from its origin, creating a hypoxic condition. The decrease in oxygen concentration alters the extracellular pH as it moves away. Therefore, such acidic and hypoxic conditions of the cancer microenvironment favor prevent them from different radiation and chemotherapeutic agents [73, 74]. Hypoxic condition in the cancer microenvironment gives rise of chemokine ligand-28 production, where this chemokine resists the immune cells to reach that environment and also assist in the angiogenesis process. The presence of this chemokine is sometimes used in the detection of late-stage cancer [5]. This chemokine is also targeted to develop targeted delivery to the cancer microenvironment to obtain an improved efficacy of chemotherapeutics [75].

2.7 Multidrug resistant system in cancer cell

Multidrug resistance is a state in cancerous cells that creates resistance of the cancer cells toward cancer chemotherapy. This resistance allowed us to survive the cancer cells against a wide range of structurally distinct chemotherapeutic agents [76]. This resistance mechanism allows us to efflux out the absorbed chemotherapeutics from the cells, thereby the drug concentration is decreased within the cell and ultimately resists for its functionality [77]. Crucial problem is that the cells will not only resist the parent chemotherapy, it will also develop cross-resistance to additional chemotherapies exerting different mechanism of action [78]. Thus, reaching of therapeutics to the cancer cell may even become ineffective because of the development of multidrug resistant barrier. Such resistance of the cancerous cells may be developed due to the mutation of the cancer cells or may be due to the intrinsic characteristics of the cancer cells. Among the multidrug resistant genes responsible for the generation of resistant, include ATP-binding cassette (ABC), a family of more than 50 genes [27, 78–80]. Access of chemotherapeutic agents to the cancer cells is limited by the ABC efflux transporter overexpression, such as breast cancer resistance proteins (BCRP/ABCG2),

MDR-associated protein 1 (MRP1/ABCC1), and P-glycoprotein (P-gp/ABCB1) [81, 82]. The cell membrane of the cancer cells is known to express these ABC bound efflux proteins that are capable of efflux out the resistance and other chemotherapeutics against the concentration gradient. Therefore, the development of multidrug resistance obstructs the delivery of the right therapy at the right time at the right concentration and in the right position. Thereby, a fraction of the drug at subtherapeutic concentration remains within the cancer cells, where the rest of the drug effluxes out via these efflux pump described above [5].

3. Conclusion

Biological barriers of the human body alter the efficacy of therapeutics via preventing their entrance or by decreasing the cellular concentration of the drug to the subtherapeutic level. Among the biological barriers, the epithelial and endothelial cells are found to be potentially involved, because cellular membranes of gastrointestinal barriers are composed of epithelial cells whereas the blood-brain barrier is composed of endothelial cells. The presence of claudins and occludin are mainly responsible to form tight junctions of the external cells, which are mainly exerting barrier characteristics of the cells. Further, the barrier features of the tumor microenvironment and multidrug resistance environment play a crucial role in the formation of the biological barrier.

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Conflict of interest

The authors declare no conflict of interest, financial, or otherwise.

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CHAPTER 5

Polymer nanogels: Fabrication, structural behavior, and biological applications

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1. Introduction

Nanogels are the combination of the favorable properties of hydrogels with colloids. As a result of this combination, nanogels can be soft, conformable, highly permeable, and stimuli responsive. Nanogels can also expose a large surface with functional groups aiming the conjugation with a range of small to large molecules including macromolecules. Nanogels can be used for targeted drug delivery and bioimaging, also having potential for water purification and catalysis [1]. Hydrogels are composed of three-dimensional networks of hydrophilic polymer chains in which drugs, polymers, and dispersed phase of liquid can be entrapped [2, 3]. These properties allow them to absorb a significant amount of water. In their composition, the presence of chemical cross-links (i.e., covalent or ionic), physical cross-links (e.g., van der Waals interactions, crystalline domains, intermolecular complexes, hydrogen bonds), or the combination of both, prevents/delays polymer dissolution in case of exposure to aqueous solutions. According to IUPAC, nanogels are a particle-gel type of any shape with an equivalent diameter of 1–100 nm. Nanogels are also considered as polymeric networks in the nano-range [4, 5].

Literature reports that different polymeric structures have been classified as nanogels, such as hydrophilic or hydrophobic cross-linked polymer nanoparticles, core-cross-linked, or shell-cross-linked micelles of amphiphilic polymers, among others [6]. Nanogels can be characterized by a hydrophilic polymer network and by an equilibrium swelling condition that coordinates the swelling of their macroscopic analogs [7].

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Nanogels present pores that are filled with micro or macromolecules that have swelling and degradation properties with flexible size, large surface area, and high water content [8, 9].

Nanogels can be used to modify the release profile of loaded drugs and release them at the target site. Controlling the particle size of the polymeric network may also be possible [10]. Due to their robust characteristics, they offer an approach to carry both hydrophilic and hydrophobic compounds [11], as well as to formulate poorly soluble drugs [12].

Nanogels are biodegradable and exhibit high biocompatibility. Nanogels have a free-flowing pearlescent network that is easily dispersed in aqueous media [10, 13, 14] and can be applied to deliver drugs by different administration routes (e.g., oral, pulmonary, nasal, ophthalmic, and transdermal) [15]. According to Guerrero-Ramírez et al. the greatest advantage of nanogels is the possibility to reduce premature leakage of the drug from the solution [16].

Nanogels may, however, be associated with limited drug-loading efficiency [17]. The major disadvantage, however, is the risk of strong interactions between the polymer and the drug which may decrease the hydrophilicity of the nanogels and as consequence can cause the structure collapse. This mechanism may lead to an irreversible entrapment of the drug molecules and enhance the hydrophilicity of the nanogel matrix [18, 19]. The presence of surfactants or monomers in the composition of nanogels formulation may also cause adverse effects [11].

1.1 Classification of polymer nanogels

Nanogels are composed of physically and chemically cross-linked synthetic polymers [20] or biopolymers (Fig. 1) [21]. Natural polymer-based nanogels are considered better candidates for drug delivery when compared to synthetic polymer-based nanogels [22].

Nanogels have been prepared using polymers of different sources, namely, natural or synthetic [23]. Chitosan, alginate, gelatin, and albumin are some natural polymers frequently used in the production of nanogels. The most representatives synthetic polymers for nanogels formation are poly(lactic acid), poly(lactic acid)-poly(glycolic acid) copolymer, poly(methylmethacrylate), poly(methylcyanoacrylate), and poly(e-caprolactone). Some authors consider that the definition of nanogel is not clear and may, therefore,

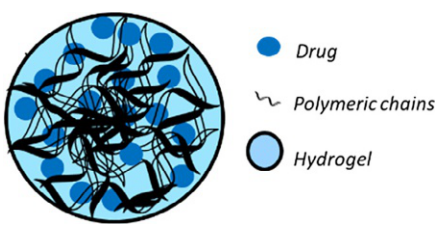

Fig. 1 Basic structure of polymer nanogels.

include polymer micelles that show a biphasic core-corona structure. However, according to these authors, this term should be applied to nanoparticles that have in their constitution a random network structure formed by both chemical or physical cross-linking [24].

In these systems, drug molecules are usually entrapped in the inner core of the particle, however, in some cases, they may be physically adsorbed or chemically linked on the surface. According to Vauthier et al. in the production of nanogels, the polymer components are firstly emulsified, followed by condensation (i.e., including precipitation, gelation, and polymerization methods). Depending on the polymer, if its molecules condensed spontaneously, the emulsification method is not needed [23]. One example of this case is the cholesterol-bearing pullulan that forms nanoparticles only by applying sonication, once self-association of the cholesteryl group occurs [25].

There are some available methods to prepare nanogels in pilot-scale for conducting clinical trials [23]. Makhlof et al. developed chitosan-hydroxypropyl methylcellulose phthalate nanoparticles. These particles are for oral insulin delivery and are formed spontaneously by ionic cross-linking [26]. Nakano et al. use the nanogel technology for coating stents, recently developed a drug-eluting stent in which poly(DL-lactide-*co*-glycolide) nanoparticles were adsorbed [27]. The hydrophilic drugs can be entrapped in these nanogels for achieving prolonged drug release when compared to a conventional stent.

2. Biomedical application

2.1 Drug and gene delivery systems

Gels are widely used in biotechnology and medicine [28]. Nanogels are delivery systems consisting of swollen cross-linked polymers. Their size is 10–100 nm and they keep their shape after dissolving in water. Polymeric nanogels are formed byphysical cross-linking and noncovalent interactions, also by using cross-linking agents for the formation of chemically cross-linked polymer nanogels. Usually, noncovalent self-assembly occurs under soft conditions. Therefore, it is attractive for encapsulation of proteins, genetic material, and biological sensitive drugs. However, self-assembled nanogels are flexible and can be reconstructed when passing through biological barriers and subjecting to various physical factors (temperature, pH, dilution) under in vivo conditions. Regardless of the field, nanogels technology uses more and more physicochemical and biological properties of self-assembled nanostructures for advanced treatments and for reducing side effects [29]. Recently, multifunctional NanoCliP nanogel was synthesized with a high porous modulus 10 times higher than that of nonporous cross-linked nanogel [30]. NanoClik gel consists of self-assembling amphiphilic polysaccharides and a chemically cross-linked network between nanogels. Nanogels are able to biodegradability and sustained release of proteins. NanoCliP gel is also able to capture proteins, liposomes, and cells on its surface.

Synthetic poly(ethylene glycol), poly(lactic acid), and poly(-Ɛ-caprolactone), and natural polymers based on polysaccharides are suitable materials for nanogels. It is known that an ideal therapeutic drug delivery system should be nontoxic, nonimmunogenic, biocompatible, with targeting potential to deliver a biologically active molecule and to reach the maximum of therapeutic efficacy with minimum toxicity. The main problem of chemically cross-linked polymeric nanogel is the presence of the remaining amount of cytotoxic cross-linking agents used in the synthesis and also the unavoidable impact of the harsh conditions of synthesis. Synthesis of these hydrogels is based on *N*-isopropylacrylamide, *N*-hydroxyethyl acrylamide, and 2-acrylamidoethyl carbamate, cross-linked with *N*,*N*-cystaminebisacrylamide or *N*-methylenebisacrylamide. After intravenous administration of these hydrogels, no tissue damage, inflammation, or morphological changes in the liver, spleen, and kidney from each treatment group were detected after analysis of blood and tissues. Synthesized nanohydrogels were biocompatible and nontoxic in vivo in rats. Thus, they can be used as drug carriers in further in vivo studies [31]. Inverse nanoprecipitation technology was used for preparing zwitterionic nanogels on the basis of poly(amidoamine)s with a size of 100 nm loaded with rifampicin as a model drug rifampicin; this showed minimal toxicity to cells [32].

The problems that limit the widespread use of synthetic chemical-linked nanogels and poor biodegradability are partially overcome if a copolymer conjugated to cleavable disulfide bond (-S-S-) or metabolic thiol glutathione, free thiols are used. One alternative way is the use of enzymes capable of making cross-linked polymers under physiological conditions, thus avoiding undesirable products and toxic agents. This approach, called "enzymatic nanogelation" to obtain stable particles (200 nm) nanogel under physiological conditions, is especially attractive in view of encapsulation of biological proteins [33].

Synthetic and natural polymers show that nanogels based on polysaccharides (chitosan, dextran, hyaluronic acid) may most widely be used as nanogels because of their nontoxicity, biodegradability, and biocompatibility. Attractive and unique delivery systems based on self-assembled polysaccharide nanogels have been reviewed by Tahara. Clinical testing in humans has shown that vaccine based on a nonionic nanogel can be administered without serious side effects [34]. The current importance of these systems depends on bioavailable and biodegradable materials based on hyaluronic acid. This is obviously related to a high biological activity and receptor-binding properties to cancer cells (CD44 and CD168). To obtain an enzyme-sensitive nanogel, modification of hyaluronic acid by methacrylate was carried out. In vivo investigations have demonstrated that Doxorubicin loaded into nanogel accumulates in cancer cells better than free Doxorubicin. Furthermore, nanogels have shown higher antitumor activity against mouse tumors H22 [35]. Another type of natural polymers (gelatin) with gum arabic was used for obtaining nanogels. Natural cross-linking agent (gum arabic) is favored slightly over other agents (glutaraldehyde, diisocyanates, genipin, carbodiimide, and oxidized polysaccharides) because of its safety. Hemo-compatible nanogels relative to MCF-7 cells in vitro

conditions were prepared by the technique of water-in-oil (w/o) miniemulsion and gum arabic as a cross-linking agent [36]. Biocompatible and hemo-compatible cross-linked nanogels from sodium alginate and gelatin were obtained using inverse mini-emulsion [37]. Curcumin-loaded nanogels from gelatin and poly(acrylamidoglycolic acid) were obtained by radical polymerization [38]. Nanogels can be prepared from heparin by self-assembly of conjugated amphiphiles and self-assembly of thiolated heparin and poly(ethylene glycol). Cross-linked heparin nanogels made by disulfide bridges and functionalized by methacrylate units display a slow release in neutral solution and a quick release under acidic conditions. Improved blood circulation ability and antitumor activity of loaded doxorubicin nanogel were observed in vivo [39]. A new method of producing hybrid biogels has been advanced for encapsulating a model protein BSA [40]. The obtained nanogel is a mixture of chitosan/polyacrylic acid with hydroxyapatite particles. The low hemolytic activity and cytotoxicity and the possibility of release allow investigators to recommend these nanogels for the regeneration and restoration of bone tissue [41].

There are other innovative techniques for producing nano-sized gels intended to the encapsulation of drugs. Nano gel consisting of pluronic and the inner core based on alginate was obtained using a simple method without chemical synthesis. The obtained nano-sized gels have shown good stability to load a positively charged protein model, lysozyme. Sustained release is achieved and the released protein retains its biological activity [42]. The simple and "green" method for producing nanogel was presented [43]. Nanogels were formed through molecular self-assembly of lysozyme and carboxymethylcellulose by electrostatic interactions. Results of in vitro cytotoxicity show that obtained nanogel loaded with a model anticancer drug (Methotrexate) has improved antitumor bioactivity [43]. Another way of obtaining nanogels is an ionomer cross-linking of complexes. This is turn can lead to the formation of particles with a small size (10–200 nm) and narrow size distribution. For example, self-assembled nanogels were obtained by cross-linking of Zn-coordinated micellar templates by removing metal ion. Nanogel loading of drug Methotrexate reduces the viability of HepG2 cells and causes cell cycle arrest [44].

It is known that cationic nanogels based on polysaccharide and modified with amine groups are very effective for intracellular delivery of proteins and DNA material, and as an intranasal vaccine delivery for pneumonia in humans [34]. Self-assembling nanogels based on cationic cycloamylose modified cholesterol and dimethylaminoethane can form nano-sized complexes with CpG DNA through electrostatic interactions. Such complexes have demonstrated the most efficient secretion of cytokine and cellular uptake in macrophage-like cells [45]. Other authors have reported the synthesis of cationic nanogels with controlled particle size for the transfection of DNA and small interfering RNA, using reversed micelles of a nonionic detergent Brij-O10 [46]. The way to overcome the lyso/endosomal barrier was presented by Maximova. It was found that the network structure of nanogel provides the unavailability of the amino group. Thus, nanogels

behave like a Trojan horse. They were protected from additional protonation and retained the properties of the buffer. This resulted in improving transfection efficiency [47]. Another interesting result was to make nanogels by complexation to carboxymethylcellulose with branched PEI. These nanogels have been used as carriers for gene delivery. In this case, the cytotoxicity of poly-branched polyethyleneimine was significantly decreased by complexation with nanogel particles. Intracellular uptake of DNA and transfection of genes were increased [48].

Combined delivery of two or more therapeutic agents is a current and innovative approach. In this case, a multiapplication therapy can be realized, and at the same time, the probability of cell resistance can be minimized. Multimodal therapy is very significant in the treatment of cancer [49]. For example, physically and chemically cross-linked nanogels were obtained in injectable form, which can simultaneously deliver IL-2, IFN-gamma, and doxorubicin to tumor regions for combined chemical and protein therapy [50]. This can best be loaded with plasmid, pVAX1-LacZ and pcDNA3-FLAG-p53, and antitumor drugs and controlled release from a chemically cross-linked biocompatible nanovectors based on nanogels [51]. New temperature/pH-sensitive superparamagnetic nanogels can deliver two different anticancer drugs (doxorubicin and methotrexate) simultaneously. Superparamagnetic properties of nanogel promoted targeted delivery to cancer cells by using magnetic field resonance. Cytotoxicity of this nanogel for cell lines MCF7 and MDA-MB-231 had appropriated to the level of carriers of drugs [52]. Multifunctional nanogels were developed by copolymerization of zwitterionic amino acids with fluorescent cross-linking agents (quantum dots). Folic acid was conjugated on the surface of nanogel as a targeted ligand [53]. Several drug molecules can be covalently bonded into the polymer chain in the polymer-drug conjugate system. A comparison of nanogels in which drugs are physically encapsulated in polymeric systems with conjugated drug-polymer systems shows that these nanogels can achieve high drug loading, sustained release, and good stability without drug leakage. All this plays a vital role in the therapeutic effect [54].

Conjugate nanogels have many important advantages: the ability to form the necessary size, a surface electric charge, the density, and a necessary functionalization. The authors lead the conjugation of retinol to dextran. These conjugates spontaneously self-assemble in water solution where they form pH-dependent nanogels. These nanogels can effectively encapsulate an antigen and achieve maturation of dendritic cells, and induce a lysosomal gap that provides an effective vaccine against cancer [55]. Conjugated quercetin to biodegradable polymer (poly (D-amino ester)) has been shown release during 45–48 h after ester hydrolysis under physiological conditions [56].

There are different approaches for the immobilization of enzymes and the preparation of immobilized catalysts. An interesting approach to immobilization nanogels on the surface was presented. This method allows an increase in the density of functional groups, also the loading of active molecules (as imaging probes, proteins, drugs, or even genes)

with the further release by various factors [57]. Another approach has been used the saving of functionality of enzyme activity and binding capacity to cellulose. For this purpose, the protein was immobilized on cellulose. The use of nanogels prevents denaturation and enzyme leaching [58].

2.2 Adhesive characteristics

It is known that the residence time of a drug on the mucosal surface can be increased if the adhesion between the polymer material and the mucosa can be obtained. As a rule, mucoadhesive polymeric carriers contain targeted sites for tissue to increase the permeability of the drug (Fig. 2).

Currently, there is considerable interest in nanogel systems as a drug delivery system for cancer treatment [59–61]. An interesting approach in development is an active platform for cancer therapy. This has been considered by Liang. It is assumed that this platform is a conjugated nanogel (hyaluronic acid-epigallocatechin-3-gallate) containing a serine protease GzmB. Hyaluronic acid was chosen as a gel component because it is a receptor of cancer cells, including cancer stem cells. Epigallocatechin-3-gallate exhibits antioxidant behavior and forms a micellar nanocomplexes with anticancer protein. Polyethyleneimine facilitates the release of GzmB from the endosome to the cytosol [62].

Recently, considerable attention in the field of smart delivery of anticancer drugs has been devoted to polymeric nanoparticles that are sensitive to various factors (pH, temperature, the concentration of glutathione) [41]. For examples, cross-linked multisensitive (oxidation, pH, temperature) biodegradable nanogels with galactose functionalization aimed at targeted delivery to liver cancer cells were obtained. The cytotoxicity of doxorubicin-loaded nanogels was evaluated using human hepatocellular cancer (HepG2) and cervical cancer (HeLa) cells. It is established that doxorubicin-loaded nanogels showed an improved delivery of doxorubicin and inhibition of hepatoma human cells (HepG2) [63]. The new design of poly(vinylalcohol) nanogels with encapsulated doxorubicin was presented. Prepared nanogels, sensitive to the extracellular and intracellular endosomal pH, cytoplasmic glutathione of tumor cells, were able to effectively intracellular release of anticancer drugs [64]. A comparison between the free drug, a synthesized polypeptide nanogels (poly (ethylene glycol) poly (L-phenylalanine-*co*-L-cystine), and the loaded doxorubicin showed an increased intratumoral accumulation and

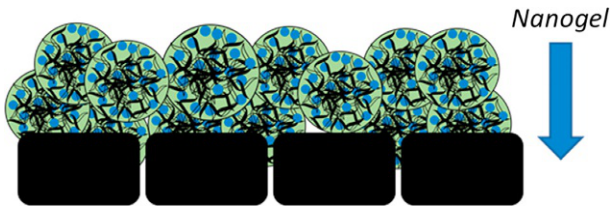

Fig. 2 Nanogels adhesive properties for mucus layers.

improved antitumor efficacy in hepatoma HepG2. Depressing of tumor cells was additionally confirmed by histopathological and immunohistochemical analysis [65].

Chitosan is a cationic mucoadhesive polymer widely employed as a carrier for intravesical delivery in connection with the strong interaction between mucin and amino groups of chitosan. However, it has some drawbacks. For this reason, it is necessary to use more tolerable mucoadhesive materials [66, 67] or apply promising approaches for the development of polymers with different ligands, improving mucoadhesive properties [68]. For example, positively charged nanogels with significant mucoadhesive properties to easily attach on the surface of the bladder were synthesized by copolymerization of acrylamide. In vitro and in vivo investigations of mucoadhesives showed that nanogels bind to UMUC3 and T24 cells [69].

This is a fact of tremendous importance to use mucoadhesive polymers in ocular therapy. They improve ophthalmic bioavailability and the prolonged effect of the drug in tissues. These requirements are usually related to the rapid washing out, and low half-life of eye drops and various barriers to the eyes. For this purpose, the authors synthesized and characterized new nanogels based on poly (butylene adipate) and *N*-succinyl chitosan having mucoadhesive properties for delivery loteprednol etabonate. Nanogels containing loteprednol etabonate were obtained by O/W emulsion technique; they showed a spherical morphology, excellent biocompatibility, and bioadhesion [70].

But in many cases, RNA-loaded polymer or lipid nanoparticles are not able to overcome biological barriers in vivo. For this purpose, hybrid (lipid-polymer) nanoparticles have emerged as a reliable and promising platform for the delivery of siRNA. For example, highly effective RNA nanoparticles for inhalation therapy using a pulmonary surfactant (Curosurf) (which plays an important role in cellular uptake due to lung-specific binding sites) and a cationic biodegradable dextran, were developed. Hybrid nanoparticles consist of a biodegradable cationic dextran nanogel core covered by a surfactant shell. In this way, it is found that the pulmonary surfactant shell of nanogel improves the stability and enhances the intracellular delivery of siRNA [71, 72].

It is common knowledge that transdermal drug delivery faces the problem of the skin barrier [73]. Effective intraepidermal delivery of a protein was achieved by a thermosensitive poly (*N*-isopropylacrylamide)-polyglycerol-based nanogels. The authors established that transglutaminase-loaded nanogels lead to the remedy of the skin barrier function [74]. The adhesive properties of a special class of self-assembled hydrogels have been improved by adding signal peptide derivatives which interact with lens epithelial cells [75]. These nanomaterials are perspective for preventing capsular opacification.

2.3 Mechanism of release

It is known that key drawbacks for drug injectable administration based on nanogels are the explosion and release of the therapeutic agent. Polymer "smart" nanoscale hydrogels

can dissolve, swell, and release therapeutic molecules. Their properties depend on temperature and pH medium. Hybrid systems consist of solid inorganic core coated with a hydrogel matrix. This can improve the stability of the structure, form a gel, and avoid the processes of radical polymerization for their preparation. Usually, pH-sensitive gels based on polyethyleneimine, poly(methacrylic acid), or poly (2-diethylaminoethyl methacrylate) are used. A simple (one-step) process for producing hydrogel for intracellular delivery of RNA and its release into the cytoplasm has been proposed [76]. These authors observed that the hydrogel coating behaves like a switch when passing from neutral to acidic medium. It is known that encapsulation and release of self-assembled peptides strongly depend on protein size and viscosity. For example, the kinetics of release of proteins (morphogenetic protein-2 and vascular endothelial growth factor) from polyethylene glycol nanogels depends on the length of polymer units (lactide and glycolide) but does not depend on the size of proteins [77]. The authors reported the synthesis of nanogel consisting of *N*-isopropylacrylamide and *N*-(2,2,6,6-tetramethylpiperidin-4-yl) methacrylamide. Properties of this nanogel are sensitive to temperature and pH. Stimulus responsive particles are functionalized using sterically hindered secondary amine and display a phase transition temperature [78].

Nanogels are frequently used for controlled drug release for several diseases (Fig. 3). Also, nanogels allow reduced release rate of drugs, provide a constant concentration of drugs under in vivo conditions for a long time, and prevent drug side effects. The creation of prolonged forms for isoniazid and rifampin is an actual problem. To solve this problem, nanogel based on poly(methacrylic acid) was synthesized. This gel shows: (i) controlled release of isoniazid and rifampin with higher activity; (ii) desired antimicrobial effect; (iii) slow release; and (iv) no cytotoxicity [79].

2.4 Sensor and bioimaging polymer systems

Noninvasive systems for imaging of biomaterial and drugs under in vivo conditions are very useful now. Nanogels with porphyrin inner core as a fluorescent probe were

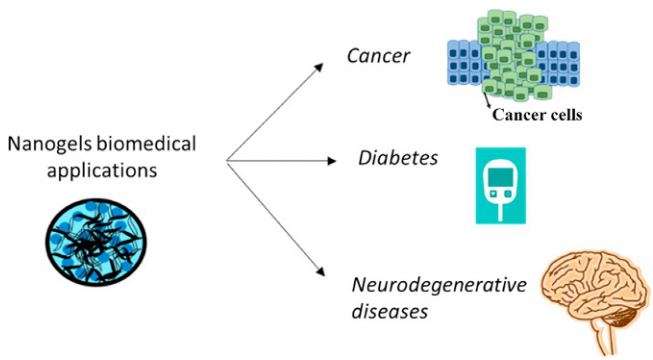

Fig. 3 Most common medical applications of polymer nanogels.

obtained. Their good biocompatibility and ability to monitor tumor tissue in vivo make them as potential drug carriers [80]. Another example of new solar nanogels for the delivery of genetic material into cells and the capabilities of imaging was represented [81]. This nanogel was a complex of the quantum dot. Gel based on heparin and Pluronic F 127 and coated with polyethyleneimine to interact with plasmid DNA. Authors described the creation of nanohydrogel loaded with QD705 and manganese; they are suitable for imaging by fluorescence tomography and magnetic resonance of cancer colon in mouse [82]. Another example of cross-linked Pluronic F127 nanogel containing a fluorescent probe for imaging of biological tissues was presented by Guo et al. [83]. For the first time, nanogels loaded with gold were obtained. It was possible to visualize 2D X-ray images of patients with lung cancer by using this injectable marker [84]. Bioimaging of protein and peptide cellular uptake has been performed by means of hybrid nanoparticles nanogels. The metals were used as cross-linking agents [85].

Sensory systems for glucose are very important for diabetic patients. Biocompatible and hemo-compatible dual-layer nanogel based on glycol chitosan/sodium alginate-poly L-glutamate-*co*-*N*-3-L-glutamylphenylboronic with encapsulated insulin and sensitive to glucose was obtained. The system is capable of controlling the release of insulin at high levels of glucose [86].

2.5 Drug delivery to CNS and penetration through blood-brain barriers

Chitosan hydrogels for delivery of Methotrexate improving brain penetration were obtained [87]. Insulin-loaded nanogels crossing the blood-brain barrier (BBB) via intranasal route were obtained for the creation of nasal spray for the treatment of neurodegenerative diseases.

Gel based on poly(*N*-vinylpyrrolidone) with carboxyl groups and a functionalized by fluorescent molecules shows improved stability, storage, biocompatibility, and ability to protect insulin from protease degradation and transport efficiency across BBB. Thus it is suitable for carrying drugs for the treatment of Alzheimer's disease [88]. Novel designed biodegradable cationic cholesterol-ε-polylysine nanogel for delivery of triphosphorylated nucleoside reverse transcriptase inhibitors with high expression of anti-HIV activity was obtained. Targeted delivery nanogel was carried out using modified nanogel brain-peptide vectors [89].

3. Conclusions

We have discussed in this manuscript various facets regarding nanogels and their applicability in the biomedical domain. In the last year, it was demonstrated that the development of new methods in the area of nanotechnology when applied to medicine can provide better ways of investigation and diagnostics with therapeutics of different diseases. The most important aspects regarding nanogels for biomedical applications are

the type of network cross-linking, the main characteristics of nanogel structures that make them appropriate for a variety of applications, bioconjugation, and encapsulation of bioactive substances and methods of preparation. In the future, nanogels will be essential as bioactive delivery carriers to improve the efficiency of drugs and the benefit of the patients.

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Conflicts of interest

The authors declare no conflict of interest.

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CHAPTER 6

Mucoadhesive nanoparticles as promising drug delivery systems

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1. Introduction

The main goal of pharmaceutical research is to design products with ensured quality to effectively treat diseases. Patient and clinician compliance is crucial to successful bench-to-bedside translation. Materials of pharmaceutical interest (MPIs) are classified into two main classes, namely, active pharmaceutical ingredients (API) and nonpharmacologically active excipients [1]. The former are entailed to trigger a pharmacological response, while the latter are incorporated into the formulation to improve its (bio)pharmaceutical properties and performance. One of the challenges in early and late PR&D pertains to the poor aqueous solubility and permeability of drugs. This property is common to approximately 50% of the APIs on the market and it represents a crucial hurdle during the stages of drug product development. Moreover, low solubility in biological fluids leads to limited absorption in the gastrointestinal tract (GIT) and limited bioavailability, the oral route is the most popular one, in addition, this route is usually associated with hepatic first-pass metabolism, chemical and enzymatic degradation in the GIT medium, basolateral-to-apical efflux by pumps of the ATP-binding cassette superfamily (ABCs), and reduced bioavailability. The most straightforward strategy to circumvent these disadvantages is the parenteral route. However, it provokes tissue damage, pain, and patient incompliance [2]. Moreover, systemic exposure often leads to adverse effects that cannot be easily controlled. The oral route is also less feasible when more prolonged release kinetics is demanded owing to the short gastric emptying and intestinal transit times. The emergence of micro and nanotechnologies together with the implementation of noninvasive and painless administration routes has revolutionized the pharmaceutical market and the treatment of disease. The interest in the capitalization of the mucus layer that covers the surface of a variety of organs by developing mucoadhesive dosage forms that remain in the administration site for more prolonged times, increasing the local and/or systemic bioavailability of the administered drug using nanotechnology is on the rise.

Theory and Applications of Nonparenteral Nanomedicines
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Aiming to overcome the main drawbacks of the oral route and to maintain high patient compliance, the engineering of innovative drug delivery systems (DDS) administrable by mucosal routes has come to light and gained the interest of the scientific community due to the possibility of changing the drug pharmacokinetics dramatically. In addition, to achieve the goal of mucosal drug administration, the development of biomaterials has been refined to fit the specific applications. Mucoadhesive materials have a strong affinity for mucosal surfaces and adhere to the surface of these tissues. Drugs may be physically or chemically bound to these mucoadhesives to increase their residence time at a specific location in the body. Additionally, the mucoadhesive effect allows for site-specific delivery of drugs to the mucosa. Mucoadhesive materials may take many forms, such as tablets for buccal delivery in situ gelling systems for ocular drug delivery, microgels for intravesicular administration, or nanoparticles targeting the GI tract [2, 3].

1.1 Mucosal features: Mucosa composition, the structure of mucin, and chemical moieties of interest

For a good understanding of the Mucoadhesion phenomenon, we must give the features of the Mucosa, especially the mucosal fluids, a special attention. From a histological point of view, the mucosa is composed of (from the lumen to the submucosa) an epithelial layer, which can be of different types and differ in mucosal foldings and roughness, thus affecting mucoadhesion. Mucus is a viscous gel layer produced from goblet cells, [1] mucus secretory cells or submucosal glands found on various mucous membranes. The presence of mucus on the membrane provides protective films for underlying epithelium. The main component of mucus is water (up to 95% by weight) [2]. Mucus is composed of, regardless the origin, cross-linked and entangled mucin fibers, sloughed cells, bacteria, lipids, salts, proteins, macromolecules, and cellular debris. Mucus is non-Newtonian, viscoelastic system comprising a tridimensional network of randomly entangled mucins (2%–5% of its mass), and presenting typical viscosity values in the range of **10–103** at low shear rate but quite variable depending on particular composition, anatomical site, and physiopathological conditions [3].

1.2 Mucus thickness and pH can vary too due to the same factors affecting viscosity

Entanglement of mucin results in the formation of dense fiber mesh in the mucus gel layer structure. An average mesh size of 100 nm was estimated from the movement of various sizes of viruses [3]. This mesh size should nevertheless be used for orientating calculations with caution, as the mucus is a very dynamic and not a static system. Moreover, this mesh size can be found just in certain microstructure regions of the mucus but not uniformly throughout the entire mucus gel layer [4]. Therefore, particle size plays an important role in the movement of particles in the mucus. The dense fiber mesh exhibits significant steric inhibition of particle movement and immobilizes particles within the mucus gel

layer. The dense fiber mesh blocks the movement of large size particles, but the enhanced ability to penetrate mucus is not linear with size reduction [5].

The mucin found within the mucus represents the primary organic component of the gel. Mucins are a group of glycoproteins coded for by MUC genes, after which the proteins are named [5a]. It is important to note that these mucins can be either membrane-bound or secreted; the latter make up the viscoelastic, shear-thinning, gel on epithelial surfaces, while the former is the loose mucus or sloppy mucus found on mucosal membranes. This type of mucus is easy to be removed by suction and shear unlike the membrane-bound mucus [4–8]. The presence of firm membrane-bound mucus on mucosal membranes results in the formation of the unstirred-water layer, the barrier for poorly soluble drugs.

Structurally, mucins are a family of glycoproteins, with molecular weights from 0.2 to >50 MDa These proteins contain a central region with an abundance of proline, threonine, and serine amino acids (the "PTS region"), of which, threonine and serine are *O*-glycosylated, giving the PTS region an extended and stiff, "bottle-brush," conformation. Some mucins contain non-PTS regions flanking this core, some of which contain cysteine residues, and therefore possess thiol groups [8a,8b]. The O-linked oligosaccharide chains adorning the PTS region are from 2 to 12 sugars long and are typically composed of galactose, fucose, acetyl-D-glucosamine, acetyl-D-galactosamine, and sialic acid. These oligosaccharides dominate the structure of mucin, comprising 50%–80% of the dry weight. There is also the presence of some N-linked oligosaccharides in the non-PTS region, though these are less abundant.

From a mucoadhesion standpoint, these oligosaccharides provide the potential for hydrogen bonding and electrostatic interaction with the carboxylic functionality of the sialic acid [8c]. The pK_a of the acidic groups in mucin is between 1 and 2.6, and as a result, mucin carries a net negative charge at pHs above this [9]. It is important to note, therefore, that the degree of ionization of the mucin oligosaccharides will greatly decrease as the pH approaches pH 1, as in the stomach. This loss of ionization will likely have a bearing on the interaction of materials with mucin. The presence of acidic saccharides and sulfate esters in the glycosylated regions of the mucin appear to have an effect on the adhesion of polymers to the surface of mucus. As previously mentioned, approximately above pH 2.6 the mucin carries a net negative charge, and given the strength of electrostatic interactions, positively charged species can interact with the mucin.

1.3 Mucoadhesion at a molecular level—Basics of mucoadhesion

Mucoadhesion can be defined as the adhesion between two materials; at least one material is a mucosal membrane. Mucoadhesion is a complex phenomenon, which is governed by many types of interaction. Thus, there is some variation in the specificity of

mucoadhesion. Several different theories have been suggested that may explain mucoadhesive interactions, but only their combination can provide a satisfactory understanding of the occurring phenomena. Understanding the essential interactions between mucoadhesives and mucosal fluids is vital for the rationale development of mucoadhesive DDS (drug delivery system). These may be specific chemical interactions, such as hydrogen-bonding, or rheological effects, as you may expect for any adhesive.

In terms of general adhesive interactions, there have been some key effects identified. For solid dosage forms, it has been suggested that the movement of water from the hydrated mucus into the dosage form will assist in its adhesion to mucosal surfaces. Several chemical interactions appear to play a part in mucoadhesion. This type of interaction will play an important role for particulates as there is not the same propensity for swelling and wetting as in the case of tablets. These chemical interactions are proposed to occur after a period of physical entanglement of the mucus and polymer. The key interactions governing the mucoadhesion at the nanoscale, which hold for many liquid dosage forms based on polymers. These are hydrogen bonding, electrostatic interactions, hydrophobic interactions, and polymer chain interdiffusion. In addition to these quite general interactions, there have been reports of materials which utilized specific chemical reactions to form covalent linkages to mucins (like disulfide bonds which are formed between polymers and cystine residues of the mucin).

Hydrogen bonding to mucins is a well-researched area of mucoadhesion. There are various sites on the mucin which are potentially hydrogen-bonding active, such as the hydroxyl groups on the oligosaccharides covering the PTS region. The ability to hydrogen-bond will be screened unless the hydrogen-bonding interaction with a mucoadhesive is sufficiently strong. It has been suggested that many of these hydrogen bonding effects are actually ion-dipole interactions between hydrogen bond donors and ionic species. This is due to the greater strength of this type of interaction, relative to straightforward hydrogen-bonding.

The presence of acidic saccharides and sulfate esters in the glycosylated regions of the mucin appear to have an effect on the adhesion of polymers to the surface of mucus. This takes us to another important aspect, which is the charge at the surface; positively charged systems are preferred to maximize mucoadhesion. One example is chitosan, which has shown to be mucoadhesive through a combination of effects, with electrostatics identified as an important interaction [10]. Chitosan and some other mucoadhesive polymers will be discussed later in this chapter.

Hydrophobicity plays a role in mucoadhesion. It has been shown that mucins can adsorb onto hydrophobic surfaces, indicating interaction. The so-called "hydrophobic effect" has been theorized to play a part in mucoadhesion. This effect is caused by the loss of entropy of water molecules as they associate to a hydrophobic macromolecule in solution. Hydrophobic molecules tend to aggregate and exclude water molecules. In addition to hydrophobic effects between mucin and mucoadhesive, the lyophilicity of mucoadhesives affects their affinity for aqueous solutions, so colloids with relatively

poor colloidal stability may be driven onto the mucosa more readily. Polymer chain interdiffusion and subsequent entanglement can aid mucoadhesion.

Specific chemical reactions on mucins may also be used to enhance mucoadhesion. Reaction with the thiol groups found in the cysteine residues of mucin has been utilized due to the high reactivity of these groups. This sort of covalent attachment has been demonstrated as a very effective method of improving the retention of dosage forms. Most commonly polymers containing thiol groups, named "thiomers" are used [10a, 10b].

Chemical modifications can be done to the polymers to enhance their mucoadhesive abilities by targeting a specific chemical reaction. For instance, the thiolated polymer ocular insert was able to adhere to the human eye through the formation of disulfide linkages [10c]. This "thiolation" is applicable to many polymers, using simple amide-coupling chemistry, which is possible in aqueous solvents [10d]. These materials are very effective mucoadhesives but are prone to oxidation that has led to the development of "preactivated" thiomers, which usually use a labile nicotinic acid moiety to protect the thiol groups from oxidation (Iqbal et al., 2012). Deamidation is a chemical process that can be done to improve mucoadhesion like in the case of pectin, commercially available pectin can be treated with ammonia thus possessing amide groups; deamination exposes amine and carboxylic acid groups and makes them available for establishing mucoadhesive bonding [11].

1.3.1 Mucopenetration at a molecular level

In contrast with the previous, mucus-inert nanosystems that avoid interaction with mucus may also be desirable. Thus, mucus-penetrating NPs may be generally obtained by conferring a hydrophilic uncharged surface. These systems are called passive systems, while active mucopenetrating systems are capable of changing either the properties of the mucus or their own properties via chemical reactions.

1.3.2 Passive mucopenetrating systems

Several polymers including poloxamers and poly(ethylene glycol) (PEG) are widely used for coating surface of particles. A type of poloxamer that is extensively used for MPP preparation is Pluronic F-127. Coating PLGA with Pluronic F-127 showed a significant improvement in particle diffusion in highly viscoelastic human mucus in the sinuses compared to uncoated PLGA. In addition, mucus penetration properties of liposomes were significantly improved when the muco-inert polymer Pluronic F-127 was incorporated [12, 13]. Modification of the surface of particles with PEG reduces interactions with both luminal components and mucus in the gut [14]. The hydrophilic and nonionic nature of PEG avoid the establishment of hydrophobic and ionic bonding, respectively, while the short chain of the polymer diminishes mucoadhesive entanglement with mucin fibers [15, 16].

1.3.3 Virus mimicking passive mucopenetrating systems

In nature, particulate systems that can easily overcome the mucus barrier are viruses. In fact, certain viruses can diffuse through mucus as fast as in saline [17]. The penetration ability of these viruses likely correlates with their special surface characteristics. Viruses exhibit a high-density charged surface of both positively and negatively charged moieties. Trivially spoken these opposite charges seem to be very busy with each other so that they do not interact anymore with the mucus. At physiological pH, they exhibit a neutral or slightly negative zeta-potential [18]. The combination of chitosan and chondroitin sulfate was used to prepare nanoparticles exhibiting equal density of positive and negative charges to mimic the virus surface. The designed nanoparticles could penetrate mucus to a higher extent compared to reference nanoparticles [19].

1.3.4 Active mucopenetrating systems

As previously mentioned, active mucopenetrating systems are capable of changing either the properties of the mucus or their own properties via chemical reactions. Mucopenetrating systems can change the properties of the mucus by having a mild mucolytic action on the mucus but not to the extent that it destroys its barrier function. Instead, mucolytic particles cleave the mucus just there where they are moving through it.

So far two different types of mucolytic systems have been developed, one that relies on breaking up disulfide bonds in the mucin and the other relies on mucolytic enzymes such as bromelain, papain, pronase, and trypsin [20, 21]. For the former, disulfide breaking agents such as *N*-acetyl-cysteine, *N*-dodecyl-4-mercapto butanimidamide, and 2-mercapto-*N*-octylacetamide are incorporated in micro- and nanocarriers [22], while in the latter the enzymes are immobilized on the surface of micro- and nanocarriers. As these enzymes are cleaving amide bonds within mucin glycoproteins in a very efficient manner, particles can much easier move across the mucus. Mucolytic enzymes can be immobilized on the polymer via amide bond formation [23]. The modified polymers are subsequently used to prepare particles. So far tested enzymes are papain and bromelain [9, 24]. In addition, Pereira de Sousa et al. could provide evidence for a significantly improved mucin mobility after exposure to enzyme-conjugated nanoparticles [24]. The modified nanoparticles showed a twofold increase in the mobility of mucin. Furthermore, bromelain revealed deeper penetration ability in mucus layer compared to papain [24].

The particles can change their own properties in order to become more mucopenetrating. The mucus gel layer exhibits a negative charge due to the presence of sialic acid and sulfonic acid substructures [9]. On the one hand, negatively charged and uncharged particles can, therefore, move easily through the mucus whereas positively charged particles are immobilized within the mucus gel layer due to ionic interactions [9, 25]. On the

other hand, however, positively charged particles show a comparatively much higher cell uptake via endocytosis than negatively charged particles [26, 27]. Thus, negatively charged particles being capable of changing zeta-potential to a positive value once having permeated the mucus and having reached the epithelium might be a promising strategy. Particles are immobilized at the absorption site once they have changed their charge into positive via ionic interactions, which is an extra advantage. The presence of certain membrane-bound enzymes at mucosal epithelium can accomplish this strategy. A recent study by Bonengel et al. reported that nanoparticles containing polyethyleneimine-6-phosphogluconic acid (PEI-6PGA) changed their zeta potential from 6.4 to +2.8 in the presence of intestinal alkaline phosphatase due to cleavage of negative charges in the form of phosphate residues [28]. In addition, Perera et al. prepared chitosan-carboxymethylcellulose nanoparticles exhibiting phosphotyrosine substructures on their surface [29]. After incubation with intestinal alkaline phosphatase, zeta potential changed from negative to positive.

Another approach was by utilizing, on the one hand, the mucus permeating properties of mucin cleaving enzymes and on the other hand, the mucoadhesive properties of thiolated systems being capable of forming disulfide bonds with cysteine-rich substructures of the mucus. On various mucosal membranes such as gastric, small intestinal, and vaginal mucosa a pH gradient from pH <5.5 on the surface to neutral at the epithelium is maintained. As thiol groups are inactive at an acid pH becoming active and forming disulfide bonds with the mucus at higher pH values, such particles can efficiently move into deeper mucus regions becoming highly mucoadhesive just close to the absorption membrane where the pH is comparatively higher. The penetration behavior of papain-conjugated thiolated-PAA nanoparticles through the mucus layer was improved by twofold higher penetration of the particles into the intestinal mucus layer in comparison with unconjugated thiolated-PAA nanoparticles [23].

1.3.5 Mucoadhesive polymers

As detailed above, the surface chemistry, charge, and size of nanosystems determine their mucoadhesive behavior. These properties are tunable in order to maximize or minimize interactions with mucus fluids present at different mucosae. When mucoadhesive systems are required, this can be simply achieved by using mucoadhesive polymers as matrix-forming materials, alone or in mixtures. However, not all the available mucoadhesive polymers can readily or easily be used to produce NPs [30] or, for instance, other non-polymeric systems may be preferable (e.g., lipid-based nanosystems). Also, surface modification can be an alternative, either by attaching mucoadhesive polymers on preformed nanosystems (covalently or by simple adsorption) or by conjugating polymers with other matrix-forming material.

Polymers are categorized according to their source; natural, synthetic, and semisynthetic. For some chosen polymers the safety, modifications done to improve their adhesiveness, and their applications will be discussed briefly in Table 1.

1.3.6 Applications of mucoadhesives

Since nanomedicine has emerged as a groundbreaking field to prevent, diagnose, and treat disease, we will discuss the applications of each nonparenteral administration route and the main therapeutic benefits of making a system mucoadhesive. Nanomedicine that only works at the crossroads of mucosal administration and nanotechnology will be addressed.

1.3.7 Oral administration

The oral route is the conventional way of drug administration, being noninvasive, painless, and permits self-administration its the route of choice for therapy of chronic maladies [60] and pediatrics [61]. In this context, the field of mucosal administration has been led by the development of DDS for oral administration which needs to be appropriately engineered to display mucoadhesive features. Nanosuspensions are probably the simplest nanotechnology applied to pharmaceutical sciences and it is entailed mainly to increase dissolution rates and, by doing so, to improve bioavailability [62–64] but this can be further improved by adding a mucoadhesive polymer to the DDS which would prolong their retention in the GIT tract and to increase the therapeutic efficacy [65] some different polymeric and lipidic nanocarriers modified with CS and PAA was designed [66], taking further one more step forward, but this time not only for physicochemical stable drugs but also for GIT sensitive drugs such as peptides and proteins [66–68] generally, the smaller the size, the more intimate the interaction between the DDS and the mucosal membrane, and the more prolonged the residence time and the drug oral bioavailability.

Pectin/liposome nanocomplexes to improve the oral bioavailability of calcitonin was produced, pectin modification increased the size of the liposomes and prolonged the residence time of the DDS in the gut over at least 6 h, reducing calcium plasma concentrations to a greater extent than the free protein solution [69] as previously discussed, positively charged polymers has an advantage over negatively charged ones in mucoadhesion. The intragastric administration of calcitonin-loaded liposomes coated or not with CS or PAA to rats and the monitoring of the calcium concentration in plasma confirmed the benefit of the positively charged surface [70]. Thus, positively charged liposomes reduced the calcium concentration to a greater extent than the negatively charged ones, the effect being more prolonged for CS-coated liposomes. However, this behavior also depends on the integrity of the intestinal wall and some works indicated that the injury of the mucosal layer in specific medical conditions such as inflammatory bowel disease (IBD) might expose proteins of the subjacent layers of the intestine that favor the adhesion of electronegative particles over the electropositive ones [71].

Table 1 Mucoadvesive polymers for drug delivery applications.

Polymer	Safety	Applications	Modifications
Alginates (*natural polymer*)	From a regulatory point of view, the US FDA recognizes ALG as "Generally Referred as Safe" (GRAS), a designation that applies to substances that are accepted as safe for alimentary use by qualified experts [31]. On the other hand, there is still some controversy regarding specific adverse effects like immunogenicity associated with ALG [32, 33]	Also, the use of alginates in depot DDS is growing [34]. Water-soluble drugs are mainly released by diffusion and poorly water-soluble drugs by matrix erosion. The release of small molecules is fast due to the fact that ALG generate nanogels with a pore diameter of approximately 5 nm [32, 35]. An additional interesting feature of ALG is that dry systems are mucoadhesive extending the residence time and the release in different mucosal tissues [36, 37]	Due to the high chemical functionality (two –OH and one –COOH per repeating unit), the chemical modification of the side chain of ALG has been extensively explored to increase the solubility in aprotic solvents [38, 39] and modify other physicochemical properties [40, 41], to attain biomimetic [42] and amphiphilic features [43] and to conjugate cell signaling ligands [44, 45]
Chitosan (*natural polymer*): CS is a linear polysaccharide which is positively charged thus its advantageous in its ability in binding to negatively charged mucin	That the toxicity of CS and its derivatives depends on the administration route	Due to the ability to establish bonds with the negatively charged mucin, CS has emerged as a fundamental mucoadhesive biomaterial [10] for a variety of administration routes such as oral ocular, nasal, inhalatory and topical [46–51]	One of the most popular strategies is grafting of thiol moieties to the chain of CS and other multifunctional polymers [52]; the process has been coined thiolation
Poly acrylic acid (*synthetic*): PAA, also known as carbomer, is a high molecular weight polymer of acrylic acid	Good biocompatibility [53]	PAA exhibits very high adhesive bond strength in contact with tissues, enhancing the mucosal penetration of drug, due to its	Copolymers of PAA and PEG. PEG has been reported to act as an adhesion promoter between PAA and mucin by linear diffusion of the PEG

Continued

Table 1 Mucoadvesive polymers for drug delivery applications—cont'd

Polymer	Safety	Applications	Modifications
		ability to control the release of drugs, PAA and, mainly its derivatives combining several substituted repeating units (e.g., poly(methyl acrylate)), have been extensively exploited as polymeric excipients for the development of conventional DDS for nonparenteral	chains into the acrylic networks and the mucin layer [54]. In another work, papain/PAA blend NPs were used to study the transport through intestinal porcine mucus compared to unaltered PAA NPs [55]
Poly(vinyl pyrrolidone) (PVP) (*synthetic*): Usually referred to as povidone, is a nonionic linear polymer comprising groups of 1-vinyl-2-pyrrolidinone	Chemically inert and essentially nontoxic for nonparenteral exposure	Although traditionally used for its mucoadhesive properties [56], PVP has been described as possessing only mild mucoadhesive properties	The mild adhesive properties of PVP seem to limit its usefulness to the development of mucoadhesive dosage forms comprising mixtures of different polymers
Cellulose (*semisynthetic*): The polysaccharide cellulose is the most abundant biopolymer in nature. Most commonly used cellulose ethers include methylcellulose (MC), ethylcellulose (EC), hydroxyethyl cellulose (HEC), HPC, HPMC, and CMC salts—calcium (CaCMC) or NaCMC [53]	The most commonly used cellulose ethers and esters for topical and mucosal drug delivery are considered as nontoxic and nonirritating materials, including some GRAS listed ones [53]	HPC presents high mucoadhesive potential. Adhesive behavior in the solid state to pig intestinal mucosa was shown to be comparable to that of several poly(acrylate)s at pH 6.8 when 300 kg/mol HPC was used [57]. HEC presents considerable mucoadhesive potential at pH 6.8 but usually lower than that of HPC and NaCMC [57]	Thiolation of HEC was done mainly by substitution of hydroxyl groups of the unmodified polymer via nucleophilic substition of a bromo-HEC intermediate with thiourea moities [58, 59]

Nano-encapsulated thymopentin has been used in the therapy of the acquired immunodeficiency syndrome (AIDS), cutaneous T-cell lymphoma/cancer immunodeficiency and rheumatoid arthritis, within poly(butyl-cyanoacrylate) (PBCA) NPs coated with CS and CS glutathione [72], All the NPs by the oral route showed a beneficial effect on immunosuppressed rats, suggesting that the absorption of the peptide was improved by just nanoencapsulation, the benefit was remarkable for systems modified with mucoadhesive polymers, especially CS-glutathione.

In another work encapsulated insulin/cyclodextrin complex within NPs made of PMAA, CS, and PEO-PPO copolymers [73] were tested in excised rat intestinal mucosa. Results showed the retention of more than 84% of the NPs even after several washings with buffer solution. it is worth stressing that reproducibility is crucial at the time of bench-to-bedside translation and that the combination of various polymers obtained from different sources added to complex production methods might challenge this task, if not preclude it. Platform of hydrophilic cyclodextrins (e.g., carboxymethyl-β-cyclodextrin and sulfobutyl ether-β-cyclodextrin) and CS NPs for the delivery of both hydrophilic and hydrophobic drug cargos [74] was explored, systems were also made more complex by the incorporation of additional components. For example, a novel DDS for the oral administration of insulin that was based on an ALG/dextran sulfate core complexed with a CS/PEG/albumin shell was developed [75]. Insulin-loaded NPs were administered to diabetic rats in doses between 25 and 100 IU/kg by the oral route. Findings indicated the sustained decrease of glycemia over 24 h with a maximum effect of 14 h after the administration. In addition, after 4 days, a dose of 50 IU/kg improved the diabetic status with a decrease of the water intake, urine excretion, and proteinuria.

Heparin, which is administered by injection within CS NPs has been investigated [76]. Even though the performance of these systems was worse than the i.v. injection, it was significantly better than that of free heparin as it augmented and delayed the anti-factor Xa effect and the plasma clotting time [76]. Other studies assessed the encapsulation of cyclosporine [77] and efavirenz [78] within polymeric NPs containing commercially available poly(methyl acrylate)s of the Eudragit series. (These copolymers have been approved by regulatory agencies for use in medical devices and usually nonparenteral pharmaceutical products [79].)

Preliminary pharmacokinetics studies showed that Eudragit RS 100 reduced the burst effect, resulting in more uniform plasma concentrations that were detectable for longer times [80]. In addition, PK parameters showed that pure Eudragit RS 100 increased the oral bioavailability of the drug with respect to pure PCL [Poly(epsilon-caprolactone)] and PCL/Eudragit RS 100 blend NPs due to increased mucoadhesion. While blend NPs showed a slight nonstatistically significant decrease with respect to PCL counterparts. While the incorporation of Eudragit increased mucoadhesion, it also decreased the release rate of the encapsulated drug. These results would indicate that in the case of blend NPs, the mucoadhesion in vivo was not strong enough to compensate for the decrease in

the release rate, resulting in a bioavailability drop. Conversely, in pure RS NPs, mucoadhesion probably prolonged the residence time and enabled the more complete release of the encapsulated drug.

Coating significantly increased the oral bioavailability of alendronate (a drug used for the prevention and/or treatment of postmenopausal osteoporosis), with respect to the free drug and uncoated liposomes [81].

LLRs are sugar-binding transmembrane proteins that are highly expressed in cells of the immune system [82] and that have been used to actively target drugs to monocytes/macrophages by the administration of sugar-modified nanocarriers [83–86]. Due to their high affinity for glycosylated structures of the mucus, a few works addressed the surface modification of different nanocarriers with soluble lectins (e.g., wheat germ agglutinin) to confer mucoadhesiveness [87, 88]. It is worth mentioning that the cost of this modification is relatively high and the scalability unlikely, what could preclude its implementation at a larger (e.g., industrial) scale.

Mucosal vaccination by the oral route has become an area that attracted a great deal of attention during the last years [89–92]. Due to its unique combination of properties, CS remains one of the key players in this research topic [93], often combined with ALG. an immune response was evaluated after oral vaccination with hepatitis B antigen (hepatitis B surface antigen, HBsAg subtype ADW2) encapsulated within ALG-coated CS NPs [94]. ALG coating was aimed to stabilize the particles and prevent immediate desorption of the antigen in the GIT medium and favored the uptake by the M cells of the Peyer's patches in the gut [95] A similar interest is found in CS for oral gene delivery [96].

1.3.8 Inhalatory mucoadhesives

Inhalatory administration has few requirements, the mean aerodynamic diameter of aerosol particles must be between the 0.5 and 5 μm to favor deposition in the deep lung, aerosol particles must have low size distribution and high reproducibility, dissolution or adhesion to the lining mucosa and appropriate drug release and permeability [97] the airways provide a very large absorption bed that can be advantageous in the treatment of pulmonary diseases (e.g., asthma) and overcoming local infectious diseases [98] due to the restriction of systemic exposure and adverse effects but also for the systemic delivery of drugs in the so-called transpulmonary route. Surprisingly, the research at the interface of nano-DDS for inhalation and mucoadhesion is elusive [99] and the reports are countable.

The beneficial effect of lecithin to increase the adhesion of liposomes to alveolar macrophages [100, 101] was shown by the group of Lehr, in two different works. Other materials were used for coating nanocarriers such as PAA, CS [66], and HPC [102]. In another work the potential of the inhalation route to treat the pulmonary form of TB was assessed, this was done by using different inhalable dry powders loaded with first-line anti-TB drugs [87, 103, 104]. This work had also another advantage; it actively

targeted the intracellular TB reservoir of the mycobacterium, alveolar macrophages [105]. Another group has successfully nanoencapsulated the anti-TB drug rifampicin within "flower-like" polymeric micelles [106] and this platform was used to develop a liquid rifampicin/isoniazid combination that showed increased oral bioavailability of rifampicin [107]. The coating of these polymeric micelles with CS and hydrolyzed GalM conferred them recognition and mucoadhesive properties [86]. These novel nano-DDS showed significantly greater uptake by macrophages in vitro [86] and good aerosolization ability [108], this approach opens new doors in treatment of this global health threat.

1.3.9 Intranasal administration

As it is the case in the inhalatory administration the nasal administration is also advantageous for having a large surface area for absorption, in addition to the high irrigation and the presence of lymphocytes and mast cells. The nasal route is advantageous as it has minimal invasiveness, painlessness, the ability for self-administration and high patient compliance [109], but on the other hand, the small dimensions and the great sensitivity to xenobiotics impose limitations to the kind of drug and DDS that can be implemented; usually, drugs administered by the nasal route must be very potent to attain therapeutic concentrations in very small administered volumes, only small volumes could be administered per nostril at each administration time and only highly concentrated systems could enable the attainment of therapeutic doses. This disadvantage has most likely precluded the bench-to-bedside translation of intranasal products [110]. The design of nano-DDS could expand the applicability of this route to other drugs [111]. However, reports on mucoadhesive nano-DDS are almost unavailable. One of the few works was developing mucoadhesive multivesicular liposomes (26–34 μm) coated with CS and Carbopol for the transmucosal (systemic) delivery of insulin [112]. The carriers contained high protein payloads between 58% and 62%. Furthermore, administration of the mucoadhesive liposomes to streptozocin-induced diabetic rats reduced plasma glucose levels in 35% for 2 days, a better performance than the uncoated ones achieved, which reduced them to a similar extent but only for 12 h. It is worth noting that this DDS was also administered by the ocular route with even more promising results; the hypoglycemic effect was observed for 72 h, ocular delivery will be discussed in the next point.

The nasal route has been capitalized in CNS drug delivery, drug delivery to the brain was always constrained by the presence of the blood-brain barrier (BBB) and the blood-cerebrospinal fluid barrier (BCSFB). For decades, these barriers prevented the use of therapeutic agents for the treatment of CNS disorders like Alzheimer's disease, stroke, brain tumor, head injury, spinal cord injury, depression, anxiety, and others. Some workers tried to alter the permeability of the BBB (e.g., with mannitol), but resulted in opening it to allow the entry of toxins, undesirable molecules, and eventually pathogens to the CNS, which resulted in potentially significant damage. Direct injection of therapeutic agents into the brain by stereotaxis is possible though this practice entails serious

drawbacks associated with the invasiveness of the procedure and the emergence of immunological side effects that limit its application in clinics. The capitalization of anatomical pathways represents an appealing approach to localize the drug release and minimize systemic exposure. It's interesting to note that the olfactory region is contiguous to the cerebrospinal fluid (CSF) tracts around the olfactory lobe [113]. Drug transport to the brain would be possible through delivery into the olfactory CSF, providing that the molecule is transported across the nasal epithelium and subsequently transported across the arachnoid membrane that separates the submucosal space of the nose and the olfactory CSF [114].

The significant increase of the bioavailability of methotrexate in the CSF with respect to plasma after the intranasal (i.n.) administration [115] was reported. Different mechanisms have been proposed for this direct passage, the paracellular one has been less investigated. The transcellular one appears as the most relevant, while in the case of drug-loaded nanocarriers, they would be internalized by the neuronal terminals of the olfactory nerve system that emerge in the brain and end at the olfactory neuronal epithelium. Thus, the i.n. administration of nano-DDS enhances the bioavailability of the cargo in the CNS. Initially, the upper limit for efficient transport across the i.n. mucosa was reported to be 100 nm though, though more recently NPs as large as 300 nm were also shown to reach the CNS [116].

Efavirenz-loaded polymeric micelles developed for oral administration was investigated [117–121] to target one of the most challenging HIV reservoirs, the CNS, employing the i.n. route. The relative exposure index in CNS was increased up to 3 times with respect to plasma [122] while the i.v. administration resulted in CNS concentrations significantly smaller than in plasma.

To overcome the disadvantage of i.n. administration regarding the restricted volume and the usage of only highly concentrated systems or potent drugs more advanced DDS comprising mucoadhesive nanocarriers as well as repetitive administration regimens could be implemented. For example, targeting of risperidone to the brain using a mucoadhesive nanoemulsion and compared it to a nonmucoadhesive one [123] was reported. Mucoadhesiveness was attained by the incorporation of CS. The brain/blood uptake ratio of risperidone was 0.617, 0.754, 0.948, and 0.054 for a solution (i.n.), a nanoemulsion (i.n.), a mucoadhesive nanoemulsion (i.n.), and i.v. nanoemulsion, respectively, at 0.5 h [123]. Formulations were prepared by spontaneous emulsification method (titration method). These results indicated the direct nose-to-brain pathway. In addition, mucoadhesive systems were more efficient than the nonmucoadhesive ones.

In another work nanoemulsions of antipsychotic drug the ziprasidone hydrochloride [124] were also developed. To confer mucoadhesiveness, systems were modified with CS. through encapsulation of thymoquinone within CS NPs prepared by the ionic gelation method with tripolyphosphate [125]. Based on maximum concentration, time-to-maximum concentration, area-under-curve over 24 h, and elimination rate

constant, i.n. thymoquinone-loaded NPs proved more effective in brain targeting compared to i.v. and i.n. thymoquinone solution. Following different strategy 32P-siRNA dendriplexes for transfection in the CNS [126] was developed too, but these nanocarriers were not mucoadhesive even though they were incorporated into a mucoadhesive gel of Pluronic F127 and chitosan or Carbopol to sustain the release. These works show the great potential this way of administration holds and probably opens the doors for the beginning of a new era in the therapy of diseases of the CNS.

1.3.10 Ocular mucoadhesives

The eye is one of the most complex and sensitive organs of the human body and it consists of three main layers, the outer coat or the sclera and cornea, a middle layer or uveal coat, and the inner coat or retina [127]. The cornea is a clear, transparent, avascular tissue to which nutrients and oxygen are supplied by the lachrymal fluid and aqueous humor. The corneal epithelium consists of 5–6 layers of columnar cells squeezed forward by the new cells. Replacement of the epithelial cells occurs by mitotic division of the basal layer every 4–8 days [127]. The conjunctiva is a thin transparent membrane, which lines the inner surface of the eyelids and is reflected onto the globe. At the corneal margin, it is structurally continuous with the corneal epithelium. The conjunctival epithelium is composed of 5–7 cell layers connected by tight junctions, which render the conjunctiva relatively impermeable. The membrane is vascular and moistened by the tear film [127] the eye is well protected from foreign materials by several efficient mechanisms forming a physical-biological barrier, such as blinking, induced lacrimation, tear turnover, nasolacrimal drainage, which cause rapid removal of drugs from the eye surface and back cornea [128]. Additionally, the blood-retinal barrier (BRB) and the extraocular epithelia represent the obstacle in the drug delivery to the choroid, retina, and vitreous. In this context, Topical DDS, especially being noninvasive way of releasing drugs in a controlled fashion directly to a specific compartment of the eye, is a promising approach, also, because they prolong the residence time of the drug in the site of action and reduce the amount of drug that is absorbed by alternative routes [127, 129]. The use of biodegradable nanocarriers has been considered a very promising system [130], among the possible strategies for ocular drug delivery, which include biocompatible viscous solutions and film-forming gels [131], liposomes [132], solid lipid NPs (SLNs) [132], microspheres [133], and medicated-contact lenses [134] though scarcely capitalized until now. In ophthalmic applications, it is convenient that particulate systems have an appropriate size, preferably within the nano-range, to prevent irritation, sensitivity toward foreign bodies, and discomfort to the patients [135]. Other factors depending on the success of NP systems for ocular drug delivery lays on the optimization of the lipophilic-hydrophilic properties of the polymer-drug system and optimizing rates of biodegradation and safety. However, great caution is required for the highly sensitive corneal/conjunctival tissues in the selection of the carriers toward eye penetration to

maximize drug transport. Different biomaterials have been used to prepare NPs, such as poly(acrylates), PLA, PLGA, dextran, ALG, collagen, hyaluronic acid, and CS [129] and their ocular application evaluated.

The most promising one is the CS, being positively charged it has the ability to develop molecular attraction forces by electrostatic interactions with the negative charges present in the eye, as it was the case with mucin as mentioned before. It was confirmed that CS NPs are up-taken by conjunctival and corneal epithelia in vivo. CS NPs cross-linked with sulfobutylether-cyclodextrin were developed to encapsulate econazole, presenting sustained drug release and better in vivo antifungal effect in rabbits compared to the free drug for 8 h [136] in different two approaches CS was used in gene delivery, to determine whether CS NPs would be suitable for intraocular use, pDNA carrying the ubiquitously expressed CBA-eGFP expression cassette was administered to adult wild-type albino mice. At day 14 postinjection, substantial green fluorescent protein expression was observed exclusively in the retinal pigment epithelium in eyes treated with GCS NPs but not in those treated with pDNA or the vehicle [137]. Moreover, no signs of gross retinal toxicity were observed, and there was no difference in electroretinogram function between NPs, pDNA, or vehicle-treated eyes. In the other approach, formulations of CS-DNA NPs were administered to rat corneas as model animal resulting in luciferase gene expression 5 times greater than following administration of PEI-DNA NPs [138]. These findings open new research avenues toward less invasive ophthalmic therapies even though they were not assessed in topical administration.

For glaucoma, two different approaches were used using NPs, melatonin, a neurohormone secreted by the pineal gland able to modulate intraocular pressure was encapsulated into PLGA and PLGA-PEG NPs [139]. Topical application of melatonin formulations caused ocular hypotension in rabbit eyes, thus emerging as an alternative approach to treat glaucoma. The maximum effect (5 mmHg), which was obtained with the PLGA-PEG formulation, occurred at 2 h and persisted up to 8 h, with a significant difference compared to melatonin aqueous solution and PLGA NPs, showing that mucoadhesion generally prolongs the contact time of a formulation with the eye surface [139] Recently, polymeric micelles of PEO-PPO [poly(ethylene oxide)-*co*-poly (propylene oxide)] have been evaluated for the encapsulation of the antiglaucoma agent ethoxzolamide [140]. But this delivery system differs in that it is not mucoadhesive, which represents a limitation for this administration route. On the other hand, the use of higher concentrations of these thermo-responsive copolymers would enable both the nanoencapsulation of the drug and the formation of a gel upon contact with the ocular mucosa. It is now well-established that polymeric mucoadhesive NPs can deliver any drug at the right time in a safe and reproducible manner to a specific anterior and posterior segment of eye at the required level.

2. Conclusion

Both mucoadhesives and mucopenetratives have shown great potential, but this potential must not be overestimated and before going for a mucoadhesive system, various points must be considered. Apart from drug-dependent aspects such as solubility, membrane permeability, and mode of action, general delivery aspects such as rapid vs sustained release properties and the mucosal route of administration have to be taken into account. Because of this complexity, a decision for a mucoadhesive or mucopenetrating system being just based on theoretical considerations might be misleading. Studies in a valid animal model for both particulate delivery systems are recommended. In most of the cases, the pharmaceutical industry shows limited interest in mucoadhesives as it is usually more economic to increase the amount of active pharmaceutical ingredient rather than designing such complex DDS. As in most cases, the improvement in bioavailability is just a fivefold increase, only few DDS show improvement above this value. This chapter should encourage scientists to further improve this approach.

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CHAPTER 7

Mucus-penetrating nanocarriers

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1. The mucus barrier

Mucus is a semipermeable and complex viscoelastic barrier that is localized in human body surfaces directly exposed to the external environment, including the respiratory tract, gut as well as the reproductive and ocular surfaces. From a macroscopic point of view, mucus is a water-based, complex, and heterogeneous gel the composition of which varies between species, individuals, and tissues [1]. The mucus barrier is composed of a secreted mucus layer and membrane-bound glycoproteins (mucins) on the surface of the cells, called the glycocalyx, which together form the mucosal surface [2, 3]. The secreted mucus layer is a mucin-based gel, where the mucin fibers are cross-linked and intertwined with each other.

Regarding the composition of mucus, mucins are the most important compounds for the properties of mucus [4], even though they make up only 5% or less of the mucus composition [5]. The 95% left are mostly water (98%) and other components such as globular proteins (0.5% *w*/*v*), salts (0.5%–1.0% *w*/w), lipids (1%–2%), electrolytes (1% w/v), DNA, bacteria, cells, and cellular debris [6, 7].

Mucins are a diverse family of glycoproteins in the MUC gene family, and so far, at least 21 MUC genes have been described [8]. They have an overall high molecular weight, which can range from 0.5 to 40 MDa [9, 10]. Their structure comprises a protein backbone that can be naked and hydrophobic in some regions or heavily glycosylated by oligosaccharides of varying size and grade of branching in other regions [11, 12]. The various types of mucins differ by the protein backbone [13], but there are some similarities, including the PTS regions rich in proline (P), threonine (T), and serine (S). These PTS regions make up about 20%–55% of the total composition of amino acids in the mucin backbone [14]. Threonine and serine residues in the protein backbone contain hydroxyl groups, on which the glycan side chains are bound through *O*-glycosylation linkages [7, 11]. These glycans (about 1–20 monomers) contain *N*-acetylgalactosamine, *N*-acetylglucosamine, fructose, galactose, sialic acid, and mannose [9, 15] and gives the mucins a negative charge, on average, due to sulfate and carboxylate residues [16]. The mucins are, on average, heavily glycosylated, but in between there are hydrophobic regions with no glycosylation, often

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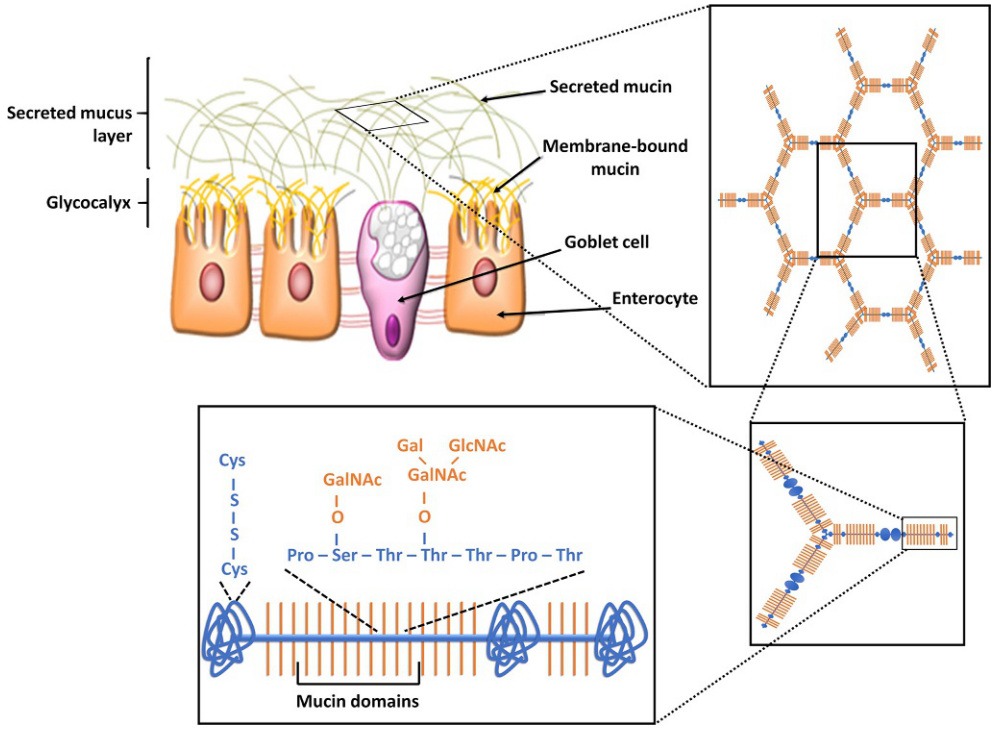

Fig. 1 Representation of the mucus layer at the intestinal epithelium and general mucin structure simplified by its protein backbone and PTS region with *O*-glycosylation linkages.

termed as naked regions [10, 17]. Hydrophobic regions contain many cysteine amino acid residues [15] that form disulfide bonds contributing to the globular shape of these regions [10]. The cysteine-rich regions are often found at the terminal ends of the mucin molecules, and large networks of mucins can therefore be formed by this cross-linkage [18]. Fig. 1 gives a general representation of the mucin structure.

Mucus structure and content directly confer some properties to the mucus. Thus, the mucus is shear thinning because its viscosity decreases with the shear rate increased [19]. The shear thinning properties of the mucus gives rise to a slippage plane as the entangled mucins are pulled apart when the mucus is subjected to shearing [17]. A slippage plane formed between the two layers allows transport of food through the intestine without damaging the epithelial cells [12]. This makes mucus an excellent lubricant and demonstrates that mucins are forming a network through low-affinity bonds and weak interactions. Linkages between mucins are being continuously broken and reformed, allowing the mucus to maintain its structure even under stress conditions [20]. These flickering weak interactions and bonds also contribute to the adhesive property of the mucus, meaning that mucus sticks to surfaces and particles [6, 15].

On the other hand, mucus also exhibits viscoelastic properties [3, 21]. In fact, mucus is simultaneously viscous and elastic. Viscosity is a measure of the resistance of a fluid to deformation when subjected to shear stress. In common terms, more and less viscous

fluids are often described as thick and thin, respectively. Elasticity is the property of a solid to return to its original state after being deformed by an outside force. Applying a small force to a mucus gel will cause deformation as the interactions within and between mucus components shift and when the force is removed, the mucus will regain some degree of its original form [17]. Since mucus is viscoelastic, it exhibits the properties of both liquid and solid substances.

2. Nanocarriers for mucosal delivery purposes

One of the greatest challenges that limit the success of nanoparticles, when administered by a mucosa of the organism, is their ability to penetrate through the mucus layer to reach the epithelium, in which the cargo should be released for effective absorption or action. Mucus represents an essential component of the body's first line of defense, designed to limit the penetration of foreign materials [22], trapping pathogens and foreign particles that are, then, removed and eliminated with the physiological turnover.

The capability of nanocarriers to diffuse through the mucus layer decreases by increasing their size. In fact, the mucus network encloses a heterogeneous mesh spacing, ranging from 20 to 1800 nm across different organs and diseases [23]. Thus, to penetrate the mucus, nanocarriers must be small enough to avoid steric obstruction and pass through the heterogeneous mesh network of mucus. As reported by Norris and Sinko, who evaluated the diffusion of polystyrene nanoparticles in porcine gastric mucin gel, translocation permeability would be importantly hampered when particle sizes reach 300 nm [24]. It has also been reported that when the mean size of particulates approaches 560 nm, they would be almost completely blocked in cystic fibrosis sputum [25]. Similar evidences about the importance of size on the capability of particulates to permeate through the mucus have been observed with self-nanoemulsifying drug delivery system (SNEDDS). SNEDDS can be defined as nanoemulsion preconcentrates or anhydrous forms of nanoemulsion. These systems are isotropic mixtures of oil, surfactant (s), and drug, which when poured in an aqueous phase under conditions of gentle agitation, spontaneously form O/W nanoemulsions (usually with a globule size of <200 nm) [26, 27]. These lipid systems can also contain cosurfactants and/or solubilizers in order to facilitate nanoemulsification or the drug incorporation in the SNEDDS. Griesser and coworkers demonstrated that SNEDDS with a size of 25 nm presented 3 times higher mucus-permeating properties than similar SNEDDS with a size of 500 nm [28].

Nevertheless, the primary mechanism by which mucus gel efficiently traps foreign particulates is the formation of polyvalent adhesive bonds [24], including electrostatic links, hydrogen and Van der Waals forces, and hydrophobic interactions [29]. Electrostatic interactions are one of the most common forms of mucoadhesion, as exemplified by chitosan, a cationic polymer, for a variety of oral and nasal drug delivery applications [30, 31]. On the other hand, using SNEDDS, the highest mucus permeation was obtained with nanocarriers with the most negative zeta potential, leading to a 2 times improvement of the mucus permeation properties in comparison with SNEDDS with a positive zeta potential [32].

In addition to negative charges imparted by the presence of carboxyl or sulfate groups on mucin fibers, there exist periodic hydrophobic naked globular regions along the mucin strands, stabilized by multiple internal disulfide bonds [3]. These hydrophobic interactions represent an important mechanism by which mucus limits the transport of bacteria and virus [6, 33]. Similarly, many types of nanocarriers synthesized from common biomaterials, such as poly (lactide-*co*-glycolide) [34], poly(anhydrides) [35], and proteins [36, 37] would be immobilized in the mucus by these hydrophobic adhesive interactions. Similarly, the composition has also an important influence on SNEDDS behavior. Thus, incorporation of long-chain lipids decreases the capability of these nanocarriers to diffuse through the mucus, compared with SNEDDS prepared with medium-chain lipids [38]. On the contrary, incorporation of Cremophor RH 40 would improve the diffusivity of the resulting SNEDDS [39]. In any case, it is important to consider that the formation of adhesive interactions between nanocarriers and the mucus may be of interest for developing mucoadhesive devices (Fig. 2). Overall, these delivery systems

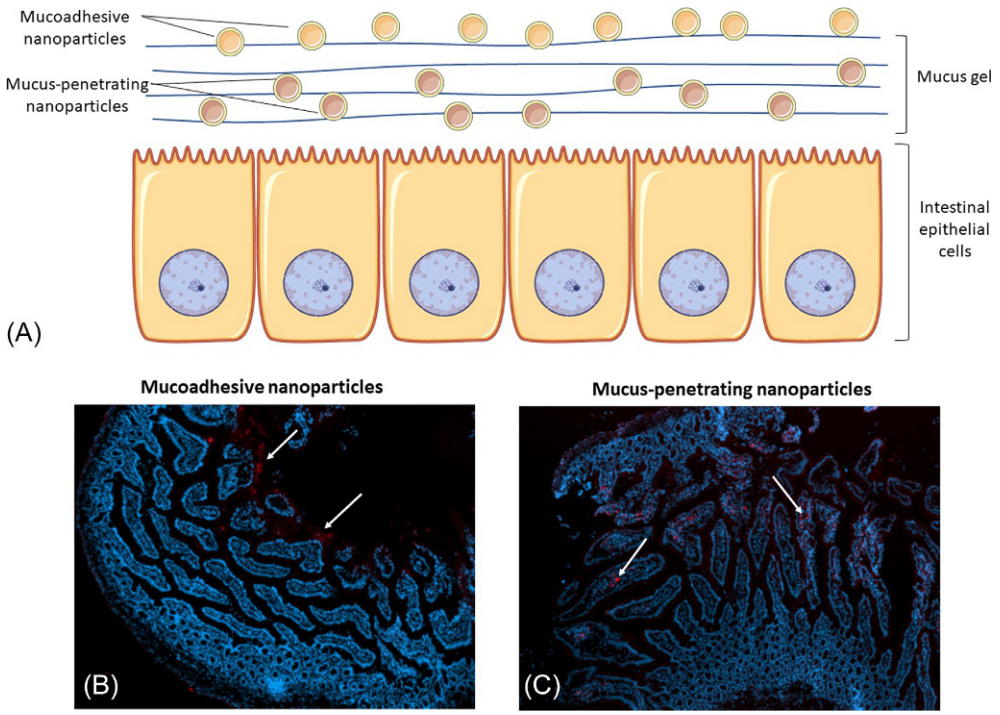

Fig. 2 Mucoadhesive nanoparticles vs mucus-penetrating nanoparticles: (A) simplified representation of the behavior of nanoparticle at the intestinal mucosa, (B) photograph of mucoadhesive nanoparticles (fluorescently labeled with Lumogen red) localized in the intestinal mucus layer, (C) photograph of mucus-penetrating nanoparticles (fluorescently labeled with Lumogen red) localized on the intestinal epithelium. Nuclei of cells was stained with DAPI (4′,6-diamidino-2-phenylindole).

offer a certain capability to improve the bioavailability of several drugs; particularly lipophilic compounds (i.e., those ascribed to Class II of the Biopharmaceutical Classification System). On the contrary, for the delivery of biomacromolecules or compounds suffering from presystemic metabolism, the presence of nanocarriers in the mucus layer, far away from the epithelium, would facilitate their destruction by digestive enzymes localized in the glycocalyx. To overcome this drawback and maximize the residence time of nanoparticles in close contact with the absorptive epithelium, the use of mucus-penetrating nanoparticles (Fig. 2) has been suggested.

3. Strategies to overcome the mucus barrier

3.1 Introduction

Mucus-permeating systems have been classified into active and passive systems [40]. Passive systems are designed to minimize the development of adhesive interactions of particles with the mucus, facilitating their transit through the epithelium. In contrast, active systems interact with the mucus and are capable of partially breaking down the three-dimensional structure of the mucus, improving their capability to permeate and diffuse into the mucus layer. In the following paragraphs, these strategies are explained in more detail.

3.2 Slippery surface strategy

The slippery approach consists of minimizing the hydrophobic interactions between the mucus components, and the generally lipophilic nature of the polymers used for the preparation of nanoparticles, by the hydrophilic coating of nanoparticles in order to produce an effective shield capable of avoiding these mucoadhesive interactions.

Strategies to avoid entrapment by the interactive mucus barrier have predominantly been focused on modifying the surface of particles by PEGylation. The presence of PEG on the surface of particulates is mainly obtained by two different approaches. The first one, a conventional bottom-up procedure with two consecutive steps, involving the functionalization of just-formed nanocarriers by physical adsorption or covalent attachment [41, 42]. The second approach is based on the synthesis of a PEG-polymer compound with either self-assembly properties or the capability to be used as material for the preparation of functionalized nanocarriers [43, 44].

In this context, Wang and coworkers [45] demonstrated that PEGylation of polystyrene is an adequate strategy for rapid mucus penetration, although low PEG MW (preferably between 5 and 10 kDa) and dense (brush) PEG surface coverage is required for an improved effect. These observations were confirmed by Cu and Saltzman, who demonstrated that for partially pegylated poly (lactide-*co*-glycolide) (PLGA) nanoparticles, the larger the PEG chain is, the more the diffusion into the mucus would be. Meanwhile, for completely coated nanoparticles, the shorter the PEG chain is, the better diffusion occurs

in the mucus [20]. More recently, Inchaurraga and collaborators described that zein nanoparticles coated with either PEG 6000 or PEG 2000 displayed improved mucus-permeating properties than when decorated with PEG 10,000 [46]. In addition, for a specific PEG, the diffusion of nanoparticles in the mucus enhances by increasing the PEG-to-zein ratio [46]. The effect of PEGylation on conferring mucus-permeating properties to nanocarriers has been also demonstrated with PLGA [45, 46] and poly(anhydride) nanoparticles [47]. Moreover, Yang et al. also produced mucus-penetrating particles, based on either PLGA or poly(*e*-caprolactone), via noncovalent coating with Pluronic F127 [48].

Other compounds that have been proposed to confer slippery properties to particulates include poly(vinyl alcohol) (PVA) [49] *N*-(2-hydroxypropyl) methacrylamide (HPMA) [50], conjugates between poly(acrylic acid) and poly(allylamine) [51], thiamine [52, 53], or dextrans [52, 53].

3.3 Mucolytic enzyme strategy

Mucolytic enzyme-decorated nanocarriers (MECS) include polymeric nanoparticles as well as SNEDDS decorated with enzymes with the capability of decreasing the elastic properties and dynamic viscosity of the mucus by cleaving amide bonds within amino acid sequences of mucus glycoproteins and, thus, breaking down its internal structure [54, 55]. As a result of this activity, tiny holes or passages through the mucus are formed and nanoparticle diffusion is significantly improved. In this context, different proteases have been proposed, including papain (PAP), bromelain (BRO), pepsin, trypsin (TRY), or proteinase K. Apart from their mucolytic activity, a further property of some proteolytic enzymes such as PAP and trypsin is their ability to open tight junctions and therefore to facilitate the paracellular transport of substances across the epithelium [56].

MECS have been prepared following different approaches. The simplest and more efficient preparative method would be based on the initial formation of a complex between the polymer and the enzyme based on either ionic interactions or covalent binding, followed by the transformation of this polymer-enzyme complex in nanoparticles by a physicochemical technique (i.e., ionic gelation, coacervation, desolvation, etc.). In this context, the first study with mucus-permeating nanocarriers was based on the formation of nanoparticles by ionic complexation at pH 4.5 between polyacrylic acid (PAA) and PAP. The resulting nanoparticles (with a mean size between 160 and 200 nm and negative zeta potential) offered an enzyme load of about 30% with a relative activity of 40%. These PAP-decorated nanoparticles showed a fivefold improvement in mucus-permeating behavior compared with unmodified particles [57]. Another strategy using PAA was the covalent binding of PAP to the polymer backbone by means of a carbodiimide derivative [58]. Once the conjugate was formed, nanoparticles were obtained by the addition of calcium, used as a cross-linker via interaction with two neighboring

carboxylic acids in PAA. The resulting nanoparticles displayed a relative enzymatic activity higher than 80%. This superior activity obtained from the covalent attachment, compared with the simple ionic complexation, would be related with an easy arrangement of the enzyme on the surface orientating toward the aqueous phase during the formation of nanoparticles with calcium [56, 59]. On the contrary, for the ionic complexation between the enzyme and PAA, papain would be part of the ionic process for particle formation yielding a more entangled arrangement with more enzyme inside the core than on the surface. In another interesting study, PAA was used as a base to immobilize papain and L-cysteine ethyl ester hydrochloride (Cys) in order to enhance enzymatic activity by increasing decomposition rates of the enzyme-substrate complex [60]. In this case, the incorporation of Cys improved by 3.5-fold the mucolytic activity of the enzyme decorated nanoparticles, compared with controls [60]. Another interesting study described the preparation and evaluation of BRO-decorated nanoparticles [61]. The enzyme content was 253 μg enzyme/mg for BRO-modified nanoparticles and the maintained enzymatic activity was calculated to be 76%.

Another possibility to produce MECS would be based on the direct attachment (via adsorption or covalent bond) of the mucolytic enzyme on the surface of nanocarriers. This functionalization approach was employed by Samaridou and coworkers, who decorated PLGA nanoparticles with various proteolytic enzymes (i.e., trypsin, papain, bromelain) via a two-step carbodiimide-coupling method [62]. Typically, enzyme loading was calculated to be between 4% and 5% with a significant maintenance of the activity. Functionalized nanoparticles with papain and bromelain exhibited a threefold higher permeability in porcine intestinal mucus compared with naked nanoparticles, whereas those conjugated with trypsin showed almost a twofold higher permeability value.

Regarding SNEDDS, and due to their inherent lipophilic character, the incorporation of hydrophilic compounds (i.e., mucolytic enzymes) is not easy. In order to solve this problem, one possible strategy is to improve lipophilicity and, thus, the solubility of the enzymes in a SNEDDS formulation. For this purpose, different strategies have been proposed including the formation of ionic complex with oppositely charged surfactants or the covalent attachment of lipophilic groups to hydrophilic groups of the enzyme in the form of acylation. In the former, PAP was conjugated with sodium deoxycholate by ionic interaction prior to its mixture with other components of the formulation [63]. The resulting SNEDD showed similar improvements in mucus diffusion than for PAP-decorated nanoparticles [56, 58]. In another study, lipophilicity of the mucolytic enzyme was increased by hydrophobic ion pairing with anionic surfactants (i.e., sodium dodecyl sulfate (SDS), sodium taurocholate (ST), and sodium deoxycholate (SDO)). The enzymatic activity of trypsin complexed with SDO, SDS, and ST in SNEDDS was 52%, 45%, and 41% respectively, of the corresponding activity of free trypsin. Enzyme-decorated SNEDDS improved mucus permeation 1.6- to 2.6-fold in comparison to controls [64].

In the latter, enzyme-palmitate conjugates of PAP, BRO, and TRY were prepared by acylation of the enzymes using palmitoyl chloride [65]. The enzyme incorporation efficiency was calculated to be between 27% (for PAP) and 39% (for TRY). On the contrary, the highest percentage of permeation in porcine intestinal mucus was achieved by introducing 5% papain-palmitate into SEDDS [65].

3.4 Combination of nanocarriers with mucolytic agents

Numerous studies have demonstrated the efficacy of mucolytic agents such as *N*-acetylcysteine (NAC) in overcoming the mucus gel barrier. NAC, and other sulfhydryl compounds, reduces the cross-linking of mucin fibers by cleaving the disulfide bonds resulting in a decrease in bulk viscosity of mucus [12, 64, 65]. This mucolytic agent has been proposed as coadjuvant treatment to improve nanoparticle diffusion through the mucus [66, 67]. Thus, in a very elegant study, Suk and coworkers demonstrated the adjuvant effect of NAC to promote the arrival of nanocarriers to the epithelium in the airways of mice with *Pseudomonas aeruginosa* lipopolysaccharide-induced mucus hypersecretion. Intranasal dosing of NAC prior to DNA nanoparticles enhanced gene expression by up to ~12-fold compared to saline control, reaching levels observed in the lungs of healthy mice [68].

More recently, the decoration of nanoparticles with NAC has been proposed to facilitate their diffusion through the protective mucus layer. Thus, Liu and collaborators described the decoration of nanostructured lipid carriers with a chitosan-*N*-acetylcysteine derivative (CS-NAC). After the instillation of these nanoparticles in the eye of rabbits, CS-NAC-decorated nanoparticles were able to penetrate through the entire corneal epithelium primarily via a transcellular route and improved by about 16 times the presence of the marker in the aqueous humor, when compared with naked nanostructures [69]. In another interesting study, a multifunctional conjugate, *N*-acetyl-L-cysteine-polyethylene glycol (100)-monostearate (NAPG) was employed to functionalize curcumin-loaded nanostructured lipid carriers. In situ intestinal perfusion studies have shown that the absorption of curcumin was directly dependent on the degree of functionalization. Moreover, in rats, the oral bioavailability of curcumin when formulated in NAPG-coated nanocarriers was up to 5 times higher compared to that of curcumin solution or when encapsulated in naked nanoparticles [70].

Other mucolytic agents that have been proposed to improve mucus-permeating properties of nanocarriers are 4-mercaptobenzoic acid and thiol derivatives. Therefore, Bouganis and collaborators demonstrated the feasibility of incorporating 4-mercaptobenzoic acid in PLGA nanoparticles or liposomes [71]. In a similar way, two novel thiol conjugates (thiobutylamidine-dodecylamine and thioglycolic-acid-octylamine) were synthesized and incorporated into SNEDDS. The resulting nanocarriers

presented superior mucus-permeating properties than conventional ones. In addition, rheological studies confirmed the mucolytic activity of thiol conjugates, which differed only by 3% from dithiothreitol (positive control) [72].

3.5 Zeta potential changing systems

Another interesting strategy to confer mucus-penetrating properties is based on nanocarriers designed with the capability of adapting their zeta potential to the environmental conditions, in order to facilitate their diffusion across the mucus layer and later anchorage on the epithelium.

As described previously, mucus possesses a negative net charge, due to the presence of sialic and sulfonic residues localized in the protein fraction of mucins [73]. Because of this, negatively charged particles can move easily within the mucus, whereas nanoparticles exhibiting a positive zeta potential are immobilized within the mucus [74]. On the contrary, positively charged nanoparticles exhibit a higher capability to bind and be taken up by cells than the negatively charged ones [73, 74]. Thus, nanocarriers capable of changing their surface charge in a controlled manner (from negative within the mucus layer to positive zeta potential values once reached the epithelium) might be a promising strategy to avoid back diffusion and to prolong interactions with cells.

To implement this strategy, certain membrane-bound enzymes of the epithelium such as alkaline phosphatase (ALP) found in many organs (i.e., intestine, lung, and the vagina) may be employed. Thus, nanocarriers containing cationic groups shielded by anionic groups in the form of phosphate esters would be attacked by intestinal ALP cleaving phosphates and by changing the zeta potential from negative toward neutral or positive [75]. This approach was evidenced by Bonengel et al., who demonstrated the charge inversion of polyelectrolyte complex nanoparticles consisting of carboxymethyl cellulose and 6-phosphogluconic acid-conjugated polyethylene imine after phosphatase treatment [76]. In another study, micelles from phosphorylated chitosan-stearic acid conjugates were evaluated [77]. In this case, micelles exhibited 6 times higher mucus permeation capacity than positively charged micelles, and their association rate to Caco-2 cells was 2 times higher compared with the association rate in the presence of phosphatase inhibitors. Similar results have been observed with SNEDDS incorporating conjugates obtained by the functionalization of lipophilic derivatives with phosphate residues [76, 77].

In contrast to these new conjugates, the use of nanoparticles based on polyphosphates has also been proposed. These nanoparticles possess interesting mucus-permeating properties. However, after enzymatic cleavage with intestinal ALP, cellular uptake increased by about 3 times, when compared with untreated nanoparticles [78].

3.6 Microorganism-like nanocarriers

It is well known that several pathogens have evolved an important arsenal of molecular strategies allowing them to penetrate the mucus layer and adhere to the epithelial, before host invasion and colonization [79]. Among others, the following mechanisms may be highlighted: (i) presence of compounds to minimize the interaction with mucus, (ii) alteration of the balance between the production and degradation of mucus, or (iii) secretion of proteinases and glycosidases [7, 78].

In order to mimic these capabilities of pathogens, one possible outcome can be the decoration of nanoparticles with biomolecules involved in the strategies developed by microorganisms for avoiding the mucus protective layer. In general, these ligands may act as a shield that minimizes the interactions of microorganisms with mucin fibers and other components of the mucus and, in some cases, may also recognize and specifically bind to receptors localized on the surface of epithelial cells. In addition, some ligands may also alter the production and/or assembly of mucin, producing mucus depletion [79–81].

Lypopolysaccharide (LPS) is an immunomodulator that specifically binds and activates antigen-presenting cells (APCs), through the Toll-like receptor 4 (TLR4) [82]. For this property, LPS and their derivatives (i.e., monophosphoryl lipid A, MPL) have been tested as adjuvants for both mucosal vaccination against different infectious diseases (hepatitis, malaria, or herpes simplex virus) and allergen immunotherapy [82, 83]. Recently, LPS from *Brucella ovis* was used to decorate poly(anhydride) nanoparticles. When orally administered to rats, the presence of LPS modified the distribution of nanoparticles within the gut displaying an important tropism for the proximal parts of the intestinal epithelium and minimizing their accumulation in the stomach mucosa. Using ovalbumin (OVA) as model allergen, these nanoparticles containing LPS elicited important Th1 and Th2 immune responses and were able to protect sensitized mice from anaphylactic shock [84]. In another work, LPS-poly(anhydride) nanoparticles were used to encapsulate a *Lolium perenne* protein extract. Again, in the challenge experiment with sensitized mice, LPS nanocarriers decreased both the levels of mMCP-1 (mouse mast cell protease-1) and the severity of the anaphylactic symptoms, increasing the survival rate of animals compared with controls [85].

Another interesting ligand is flagellin. Flagellin is the major subunit protein that forms the bacterial flagellum, providing motility and increasing adhesion to different microorganisms such as Salmonella [84, 85]. Thus, Salmonella-like nanoparticles were produced by the functionalization of poly(anhydride) nanoparticles with flagellin of Salmonella Enteritidis [86]. When administered orally, these nanocarriers displayed an important capability to reach the surface of the epithelium, mainly in the ileum of laboratory animals. Interestingly, the distribution profile of these nanoparticles within the gut correlated well with the described colonization profile for Salmonella enteritidis, including

a broad concentration in Peyer's patches [87]. Using OVA as the model antigen, Salmonella-like nanoparticles induced a strong and balanced secretion of both IgG2a (Th1)- and IgG1 (Th2)-specific antibodies. In addition, these nanoparticles were able to induce a much stronger mucosal IgA response than control nanoparticles [88].

On the other hand, a biomimetic approach would be also the use of mannose and certain mannose glycans with the ability to promote the interaction of some microorganisms (e.g., *Listeria monocytogenes*, *Leishmania donovani*, Enterobacteriaceae, or Bifidobacterium) with cells of different mucosal epithelia [89, 90]. This mechanism of interaction is related to the high binding affinity of mannose residues to the so-called mannose-binding lectins (or mannose receptors). In this context, mannosylated nanocarriers have demonstrated a high capability to reach and remain adhered to the surface of the ileum epithelium of rodents [91]. In mice, these nanoparticles containing an antigenic extract of *Brucella ovis* when orally administered offered an important degree of protection against an infective challenge (2-log reduction in spleen CFU compared with the control) [92]. In a more recent study, mannosylated nanoparticles obtained from a polymer conjugate between mannosamine and a poly(anhydride) displayed a diffusion coefficient in intestinal pig mucus 35-fold higher than for PLGA nanoparticles. This higher diffusivity in mucus was evidenced in vivo by an important capability of the nanoparticles to reach, to a large extent the intestinal epithelium of animals, including Peyer's patches [93]. When these mannosylated nanoparticles (containing a peanut extract) were administered to peanut-sensitized animals, they offered both less serious anaphylaxis symptoms and higher survival rates than controls, confirming the protective effect of this formulation against the challenge [93].

On the other hand, another biomimetic approach may be to imitate the behavior of many viruses such as the Norwalk or polio virus that are capable of diffusing through a mucus layer as fast as in water [10]. This virus possesses in its surface an important number of both positively and negatively charged groups leading to an overall net neutral surface charge and, thus, minimizing the virus entrapment in the mucus layer [10, 94]. This strategy was evidenced by Laffleur and coworkers employing synthetic particles of near-neutral surface charge (+0.9 mV), based on the combination between polyacrylic acid (PAA) and polyallylamine (PAM). The resulting nanoparticles displayed improved permeation in the mucus [51].

4. Conclusions

Patients, in general, perceive mucosal routes of administration (i.e., oral, ocular, nasal, etc.) as more comfortable and convenient than parenteral ones, particularly, for chronic medication regimens. However, these routes are faced with several hurdles that limit the absorption and bioavailability of many biologically active compounds.

The use of nanocarriers has been proposed to promote the bioavailability of drugs. In principle, some of these delivery systems may be very effective in protecting the payload against premature inactivation by the action of pH conditions and/or the presence of enzymes. On the contrary, these nanocarriers are in general not able to pass the protective mucus layer lining the absorptive epithelium and, then, are eliminated from the gut mucosa due to the physiological mucus turnover. In order to solve this barrier, the use of nanoparticles with mucus-permeating properties has been proposed. These developments include the application of bioinspired procedures mimicking the key features of microorganisms and a combination of nanocarriers either with agents able to disrupt the structure of mucus or with hydrophilic compounds in order to minimize the interaction with the hydrophobic domains of mucus.

There are still relatively few studies on the use of these nanocarriers with enhanced mucus penetration. In general, these studies cover the preparation and evaluation of their diffusivity in mucus, although very little information can be found about their real potential to promote the bioavailability or the efficacy of a drug. Another important point that sometimes is forgotten during the development of nanocarriers for mucosal delivery is the evaluation of the influence of the payload on their mucus-permeating properties. The incorporation of a biologically active molecule may significantly modify the physicochemical properties of empty nanocarriers and, hence, negatively affect their ability to reach the epithelium. In any case, the design of these new generation of transporters can be an interesting approach to solve some of the problems associated with the mucosal delivery of biomacromolecules and other sensitive compounds.

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CHAPTER 8

From the nose to the brain, nanomedicine drug delivery

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1. Introduction

In the present century, the central nervous system (CNS) disorders are expected to escalate enormously owing to a projected increase in human life. Among the numerous CNS disorders, neurodegenerative ailments are the most cumbersome and afflict humankind globally [1, 2]. Although, the etiology of neurodegenerative diseases is intricate but it is generally attributed to the decline of neuronal efficacy with increasing age. Alzheimer's disease, Parkinson's disease, Huntington's disease, Prion disease, and spinal muscular atrophy are the major neurodegenerative ailments [3]. The pathophysiology of Alzheimer's, Parkinson's, and Huntington's disease is not fully understood despite the extensive research conducted [1]. Numerous obstacles, such as the blood-cerebrospinal fluid barrier and blood-brain barrier (BBB) must be triumphed for the adequate delivery of drug molecules to the CNS to ensure an effective treatment of neurodegenerative diseases. Over the past few decades, nose-to-brain delivery approach for the administration of therapeutic agents to the brain has acquired enormous importance [4]. Numerous studies have indicated the potential of intranasal delivery for the administration of drugs, peptides, proteins, gene vectors, and mesenchymal stem cells in successfully bypassing the aforementioned barriers [5, 6]. Intranasal drug delivery has plenty of merits for instance, bypassing the blood-brain barrier (BBB) [7], avoidance of first-pass metabolism and systemic dilution, and provision of delivering small doses, which mitigates the drug toxicity [8]. Nonetheless, nose-to-brain drug delivery has been associated with some drawbacks as well for instance, the restricted surface area of the olfactory epithelium, nasal congestion, harm to the nasal mucosa, low epithelial permeability, enzymatic degradation, and the poor absorption owing to mucociliary clearance [9]. Numerous approaches such as employment of the absorption enhancers and mucoadhesive polymers to extend the residence time of in the nasal cavity have been adopted to escalate the penetration of drugs into the nasal cavity [10]. The emergence of nanotechnology in the drug delivery field has offered a novel choice in the cure of neurodegenerative diseases because of

Theory and Applications of Nonparenteral Nanomedicines
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site-specific targeting. The drug delivery to the brain via nasal passage using nanocarriers allows appropriate brain targeting resulting in improved bioavailability [8, 10]. In this chapter, we provide comprehensive details of the current treatment of PD, AD, and HD. In addition, this review also emphasizes on the recent studies aimed at the administration of nanomedicine through nose-to-brain delivery to improvise the current therapies available for CNS disorders.

2. Parkinson's disease

Parkinson's disease (PD) is a progressive, chronic, and age-dependent neurodegenerative disease afflicting approximately seven million human population worldwide [11]. In 1912, Friedrich Heinrich Lewy a German pathologist reported the presence of neuronal cytoplasmic inclusions in various parts of the brain [12] and was termed as Lewy bodies. Later on in 1919, Tretiakoff witnessed the depletion of neurons in the substantia nigra pars compacta of the midbrain in PD patients. In addition, the role of dopamine and its deficiency in the basal ganglia was known in the 1950s [12]. The chances of PD in a human population tend to surge with aging [13, 14]; the projected PD incidences increase from 41 cases per 100,000 individuals in the 40–49-year age group to 1903 incidences per 100,000 persons in elderly people over 80 years [14–18]. The prevalence of PD in males is significantly higher, with an estimated male-to-female ratio of about 1:5 [19]. The possible reasons for this gender-dependent incurrence PD might be a more pronounced work exposure in men compared with women and second, a cushioning effect on neurons imparted by estrogens in women, as well as X-linked genetic factors [19]. Sporadic PD is induced by a combination of genetic as well as environmental factors indicating a relationship between the rise of PD and the emergence of industrial developments [20]. The main symptoms of tremor at rest, bradykinesia, and muscle rigidity are ascribed to the depletion of dopaminergic neurons, which potentially dissipate the quality of daily activities in PD patients [14, 21–25]. If untreated, the intensifying symptoms of PD gradually lead to chronic disability and thereby mitigating the quality of life (QoL) in PD patients [26].

3. Alzheimer's disease

Alzheimer's disease (AD) is another prominent neurodegenerative disease associated with dementia and developing generally with aging. AD is accounted as a primary cause of dementia and the symptoms appear in three stages namely mild, moderate, and severe. Usually, AD begins with a loss in short-term memory progressing to cognitive impairment, psychiatric issues, behavioral fluctuations, problems in the coordination, and finally leading to a premature death [27, 28]. The estimated number of dementia patients globally is 24 million with a projection to be doubled by the year 2040 [29]. AD is also

a chief cause of mortality in Europe and is also ranked sixth in the United States [30]. A few of the genes involved the manifestation of familial AD are presenilins-1 and -2, alpha-2 macroglobulins, and apolipoprotein E (ApoE) [31, 32]. Numerous theories have been postulated regarding the pathogenesis of AD at the micro-level; primarily based on the functions of "amyloid, cholinergic, tau, glutamate, and oxidative stress hypotheses" [33, 34]. In addition, there are also several documented theories on the pathogenesis of AD pertaining to the formation of neurofibrillary tangles (NFTs) and amyloid-β plaques along with oxidative damage, inflammation, iron dysregulation, and cholesterol metabolism. The neurodegeneration particularly observed in PD is correlated to two prominent processes namely the buildup of extracellular senile amyloid plaques and the formation of intracellular NFTs owing to the hyperphosphorylation of tau proteins present in neurons.

4. Huntington's disease

Huntington disease (HD) is an infrequent neurodegenerative disorder of the central nervous system identified by involuntary choreatic movements, behavioral and psychiatric manifestations, and dementia [35]. Incidence in the human population is estimated at 1/10,000–1/20,000 with an average age of onset is between 30 and 50 years. In rare scenarios, symptoms appear before entering the 20s coupled with behavioral issues and cognitive impairment during early education (Juvenile Huntington's disease (JHD)). The chorea steadily progresses to all body muscles and thus deteriorating the plight [36]. Majority of psychomotor functions become extensively impaired and patients can experience psychiatric symptoms and cognitive collapse. HD is considered as an autosomal dominant hereditary disease induced by an extended CAG repeat (36 repeats or more) on the short chain of chromosome 4p16.3 in the Huntingtine gene. The widen the CAG repeat, the rapid would be the onset of disease and repeat often surpasses 55 in case of JHD. Diagnosis is primarily dependent on the symptoms and is authenticated by DNA analysis [37]. Differential diagnoses involve other possible means of chorea such as iatrogenic disorders. Occasionally, phenocopies (clinically identified cases of HD without any genetic alterations) have also been reported. Prenatal screening is made possible using chorionic villus sampling or amniocentesis. Preimplantation diagnosis with in vitro fertilization is offered in several continents and territories [38]. HD cannot be cured, therefore, the treatment must be multidisciplinary focused on mitigating the symptoms with an intention to enhance the quality of life. Chorea is usually controlled using dopamine receptor antagonists or depleting agents and medical as well nonmedical support would be needed for depression and aggressive behavior. As HD intensifies, patient becomes entirely dependent on others to lead the daily life thus necessitating the full-time care until death. The most commonly observed trigger of death is pneumonia succeeded by suicide [39].

5. Therapeutic targets and treatment

Hitherto, there is no efficacious treatment to impede the advancement of neurodegeneration in PD. The present therapy relies primarily on pharmacological replacement of smear dopamine, and other therapeutic agents [40]. Apart from this, the alternative therapeutic strategies contain physiotherapy and deep brain stimulation [41]. Substances such as l-DOPA with peripheral DOPA decarboxylase inhibitors are generally employed to manage PD predominantly by escalating the brain dopamine content while diminishing peripheral untoward effects [42]. Nevertheless, l-DOPA is usually prescribed in combination with other complementary therapeutic agents namely monoamine oxidase-B inhibitors (rasagiline, selegiline), catechol-*O*-methyltransferase inhibitors (entacapone, tolcapone), and dopamine agonists (rotigotine, bromocriptine, pergolide, lisuride) [43, 44]. Nonetheless, the prolonged usage of l-DOPA and other drugs can lead to certain implications for instance involuntary movements. In addition to dopamine, more neurotransmitters namely acetylcholine, serotonin, noradrenaline, glutamate, and adenosine are also convoluted in PD and contribute to its manifestations. Therefore, other therapeutic agents such as adenosine A2A receptor antagonists (tozadenant, istradefylline), *N*-methyl-D-aspartate (NMDA) glutamate receptor antagonists (traxoprodil), metabotropic glutamate receptor 5 (mGluR5) allosteric modulators (mavoglurant, dipraglurant), and serotonergic agents (eltoprazine, a partial agonist at 5HT1A/1B receptors) aiming at the aforementioned neurotransmitters have been developed to ease the manifestations and untoward effects emanated amid the management of PD [45, 46]. In addition, novel approaches involving genomic and cellular methods to revive striatal dopamine levels and innovative methods such as direct nose-to-brain delivery of existing drug molecules are currently being investigated [39].

AD management involves either the enhancement of cholinergic transmittance in the central nervous system (CNS) or to deter the excitotoxic effects introduced by the hyper-activation of NMDA-glutamate receptors present in some segment of the brain [47]. Similar to PD, these approaches render interim symptomatic comfort by mitigating the magnitude of cognitive retardation. The drugs used to treat AD are (a) cholinergic activators; (b) glutamate (NMDA) antagonists; and (c) miscellaneous drugs [32]. Cholinergic activators such as tacrine, rivastigmine, donepezil, and galantamine hinder acetylcholine esterase (AChE) enzyme and provide temporary relief by imparting some disease-moderating effects [48, 49]. Alternate therapies include antioxidants and vitamin supplements [50, 51], stem cell therapy [33], lipid-lowering drugs, selective phosphodiesterase (PDE) inhibitors, inhibition of β-secretase and γ-secretase and Aβ aggregation, inhibition of tau hyperphosphorylation and intracellular NFT hormonal therapy, antihypertensive therapy, selective phosphodiesterase (PDE) inhibitors, inhibition of β-secretase and γ-secretase and Aβ aggregation, inhibition of tau hyperphosphorylation and intracellular

NFT [33, 50, 51], transition metal chelators [33], nonsteroidal antiinflammatory drugs (NSAIDs) [52], insulin-resistance drugs [52], and brain-derived neurotrophic factor (BDNF) [53]. Administration of insulin using intranasal route is so far the only treatment demonstrated a robust restoration of memory and other functions in Alzheimer's disease without significantly modifying the blood levels of glucose or insulin [54] along with an enhancement in brain cell energy (ATP) in humans [55, 56].

6. Obstacles and strategies for brain targeting

As aforementioned, a drug molecule employed in the treatment of a neurodegenerative disease must cross numerous barriers such as the blood-brain barrier (BBB) and the blood-cerebrospinal fluid barrier (BCSFB) to initiate an effective therapeutic response [9]. The anatomy and physiology of the BBB and BCSFB have been thoroughly documented [3, 54, 55]. The BBB is comprised of a specific system of capillary endothelial cells connected using tight junctions, carrier proteins, and enzymatic barriers that impede the penetration of noxious entities into the brain tissues to preserve the brain's internal environment [54]. Nevertheless, the BBB also possesses several endogenous carriers, permitting the pivotal nutrients and minerals to enter, while reducing the permeation of other external entities including drugs [56]. In addition, the BBB also separates the circulating blood from the brain's extracellular fluid (ECF). The physicochemical characteristics of drugs such as lipophilicity and molecular weight govern the magnitude of crossing the BBB. Usually, lipophilic drug molecules having low molecular weight (400 Da) not ionized at body pH are the candidates to overcome the BBB via diffusion [57]. Vital substances namely amino acids, neuropeptides, and hexoses usually require particular carriers for their penetration into the brain [57], whilst proteins and peptides can triumph the BBB using saturable transport systems [49]. Despite the crossing the BBB, the brain has additional protective measures such as protein transporters (P-glycoprotein (P-gp)) and multidrug efflux pumps to neutralize the possibility for systemic brain exposure [54, 58, 59]. Location of efflux transporters on the BBB is usually attributed to the drug expulsion from CSF of brain. The BCSFB consisted of choroid plexus epithelial cells connected by tight junctions not by the endothelial cells encompassing a dormer blood capillary system. The BCSFB serves as a tangible barrier that minimizes the entrance of molecules and ions in the brain [57]. The presence of brain's defensive system has triggered the development of numerous approaches to triumph this defensive shield in order to escalate the bioavailability and targeting of various therapeutics in the brain [60]. For instance, intrathecal (BBB disruption) delivery, intraparenchymal, chemical alterations, usage of prodrugs, and conjugation of a drug molecule with a ligand or an antibody are important approaches used to cross the BBB [61]. These approaches to cross the BBB are well documented and elaborated in detail [54, 60]. In the recent times, intranasal (i.n.) drug delivery has been acknowledged as a novel

noninvasive, commendable substitute to parenteral, and oral delivery drugs to the CNS. The intranasal (i.n.) drug delivery has the capability to revamp the treatment protocols of PD, AD, HD, and several other brain disorders [62].

7. Intranasal (nose-to-brain) drug delivery

The intranasal delivery affords a noninvasive method of evading the BBB and thereby facilitating the prompt delivery of drug molecule to the CNS [10, 56, 63]. The prominent merits and demerits of intranasal drug delivery are depicted in Table 1. Several authors have well reviewed the anatomy and physiology of the nasal cavity [55, 64–66]. The nasal cavity is comprised of two regions namely the respiratory region and the olfactory region predominantly accountable for drug assimilation into the blood and brain. The respiratory region is the primary location for achieving the systemic drug delivery. Several theories have been described for the absorption of drugs administered using the nose. Nonetheless, the most prominent are the transcellular and transcytosis pathways by vesicle carriers and the paracellular passive absorption [67]. Generally, a low molecular weight and lipophilic drug is promptly and effectively assimilated from the nasal region and thereby can accumulate in the brain by overcoming the BBB [68]. The olfactory region and upper segment of the nasal cavity is the principal platform for the direct penetration of a drug molecule into the brain using the olfactory and trigeminal neural pathways [55, 69, 70]. Nevertheless, numerous parameters such as a

Table 1 Prominent merits and demerits of nose to brain drug delivery.

Merits	Demerits
1. Noninvasive and readily available to brain	1. The whole dosage should be administered in a small volume range between 25 and 200 μL
2. Probable direct transmittal to CNS by circumventing the BBB	2. Prompt expulsion of a drug molecule from the nasal mucosa by the mucociliary clearance system
3. Provision of direct delivery of drugs into both systemic circulation and the CNS	3. Degradation of drugs nasal enzymes cytochrome P450/peptidases/proteases
4. Avoidance of acidic or enzymatic degradation of drugs in gastrointestinal tract	4. Limited available surface area for absorption compared to that of the gastrointestinal tract
5. Evading the hepatic first pass metabolism	5. Nasal congestion may induce irritability and obstruction in drug absorption
6. Prompt absorption and rapid start of action	6. Limited penetration for lipophobic drugs requiring the use of absorption enhancers
7. Enhanced bioavailability thereby lowers the dose of drugs	7. Expulsion of systemically assimilated drugs via natural clearance pathways
8. Reduction in the chances of overdose	8. Nose-to-brain route of delivery is not appropriate for all drugs
9. Provision of self-administration and dose adjustment	9. Individual fluctuations leading to failure

molecular weight above 20 kDa [71], enhanced drug hydrophilicity [72] and the extent of drug ionization [73] may impede the assimilation of drugs into the CNS via the intranasal route. Apart from these aforementioned factors, enzymatic degradation and active efflux transporter pumps (P-gp) mounted on the olfactory epithelium can also interfere the transport of drugs [74]. In addition, several other conditions such as nasal congestion, injury of nasal mucosa, and mucociliary clearance may also interfere with the nose-to-brain formulations. Consequently, numerous approaches such as the usage of absorption enhancers [65, 75] and the use of mucoadhesive polymers [10] have been adopted to foster the penetration of drugs into the nasal mucosa.

Hitherto, the fundamentals of absorption of drugs via nose-to-brain delivery are not fully comprehended. However, the assimilation of drugs via this route is primarily ascribed to two pathways namely systemic and olfactory or trigeminal nerves pathway (Fig. 1). It is speculated that usually a combination of these two pathways is involved. Nonetheless, one pathway may outweigh the other based on the composition of formulation, physiochemical characteristics of drugs, and the type delivery device

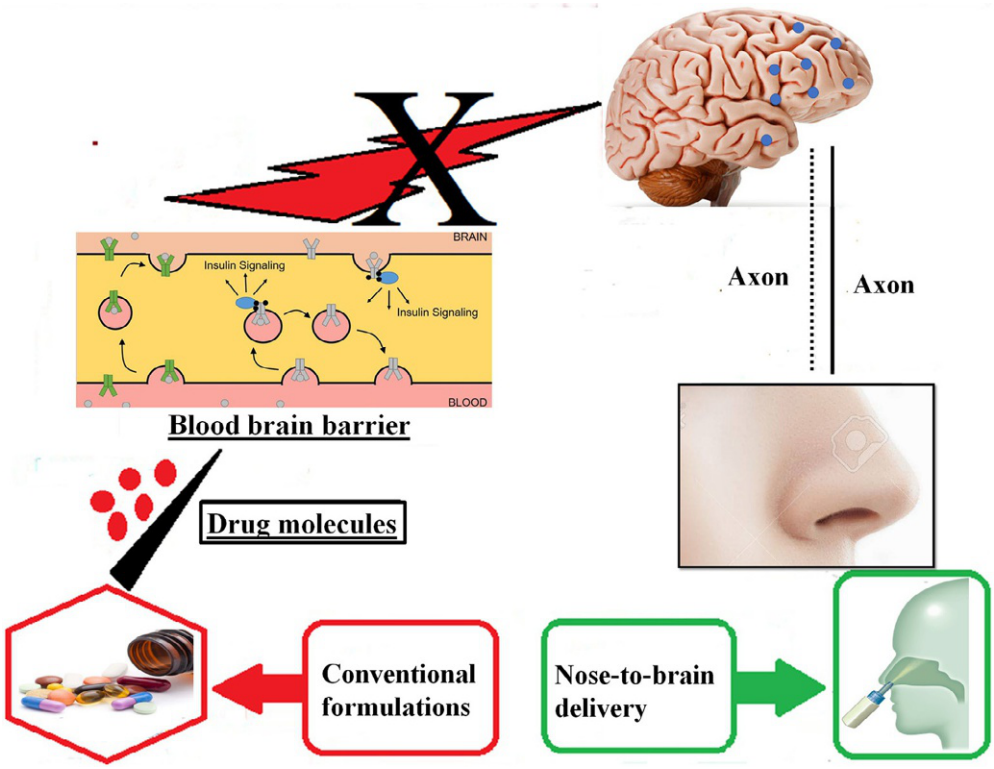

Fig. 1 Illustration of various pathways to brain following the nose-to-brain drug delivery.

employed for intranasal administration [55, 63, 66]. The systemic pathway involves the direct absorption of a drug through the nasal mucosa into the blood followed by penetration into the brain via the BBB [55]. The olfactory or trigeminal nerve pathway originates in the brain and abolishes in the nasal cavity at the olfactory neuroepithelium or respiratory epithelium, respectively [75–77]. The olfactory neuroepithelium or respiratory epithelium is the only segments of the CNS and consequently is exploited for the direct noninvasive entry of drugs into the brain. The possible mechanisms that govern the direct nose-to-brain drug delivery are namely intracellular transport-mediated route and extracellular transport-mediated routes [75, 78]. The intracellular transport-mediated route is a comparatively restricted mechanism and relies on axonal transport and requires significant time for the intranasally delivered drugs to access the olfactory bulb. The olfactory neurons present in the olfactory epithelium engulf drug molecules via endocytosis [9, 77, 79]. In the contrary, the extracellular transport-mediated route supports the swift entry of drugs into the brain within a few to 30 min [78, 80, 81]. Apart from these aforementioned pathways, a drug administered using nose-to-brain route drugs can also access the CNS using CSF and lymphatics.

8. Nanotechnology tools for delivery of neurotherapeutic agents via intranasal route

The appellation nanotechnology includes all materials, processes, and the systems exhibiting a novel physical, chemical, and biological properties or phenomena due to the size range in the nano-region [82]. Nanotechnology has been employed to reinforce the performance of the treatment regimen intended for neurodegenerative diseases via nose to brain [82]. Nanotechnology can render different pharmacokinetics profiles and improve the delivery of various diagnostic and therapeutic agents [83]. The chief advantage of nanotechnology-based drug delivery systems includes targeted drug delivery for specific action in the CNS by triumphing the obstacles in drug permeation and consequently boosting the bioavailability and therapeutic efficacy of anti-PD, anti-AD, and anti-HD agents. The nanoparticles (NPs) employed for therapeutic interventions are generally solid colloidal particles with a size range between 1 and 1000 nm [3, 57]. Besides size reduction, NPs can cushion the thermolabile therapeutic molecules such as DNZ, proteins, and peptides and can exhibit the site-specific drug delivery. In addition, these NPs ensure the therapeutically effective drug concentrations in the blood plasma by enhancing the solubility, half-life, and permeability of drugs. The intensity and incidence of untoward effects are minimized owing to precise and accurate targeting to induce therapeutic responses even at low drug concentrations [32, 84]. Lastly, NPs may safeguard the embedded drug from the enzymatic and/or chemical degradation and from extracellular transport by P-glycoprotein efflux, thus enhancing their availability in the CNS [10]. Nanoparticulate systems are

predominantly categorized into polymeric nanoparticles, lipid-based solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs), micelles, liposomes, carbon nanotubes, nanospheres, dendrimers, nanocrystals, and nanoemulsions (Fig. 2). Table 2 highlights the documented research work conducted on the fabrication of nanoparticulate systems intended for a therapeutic intervention in neurodegenerative disorders via direct nose-to-brain delivery.

8.1 Polymeric nanoparticles

Polymeric NPs are solid colloidal particles in which drugs can be embedded, entrapped, dissolved, or chemically adhered to the polymer matrix [110–112]. The diameter of polymeric NPs is generally larger than those of micelles, with a usual size range between 100 and 200 nm, and possesses an elevated polydispersity index [113]. Polymeric NPs exhibit marvelous sustained/controlled drug release profiles, biocompatibility, biodegradability, and stability. In addition, they also possess negligible toxicity and immunogenicity and are economically feasible to be manufactured [114]. Numerous

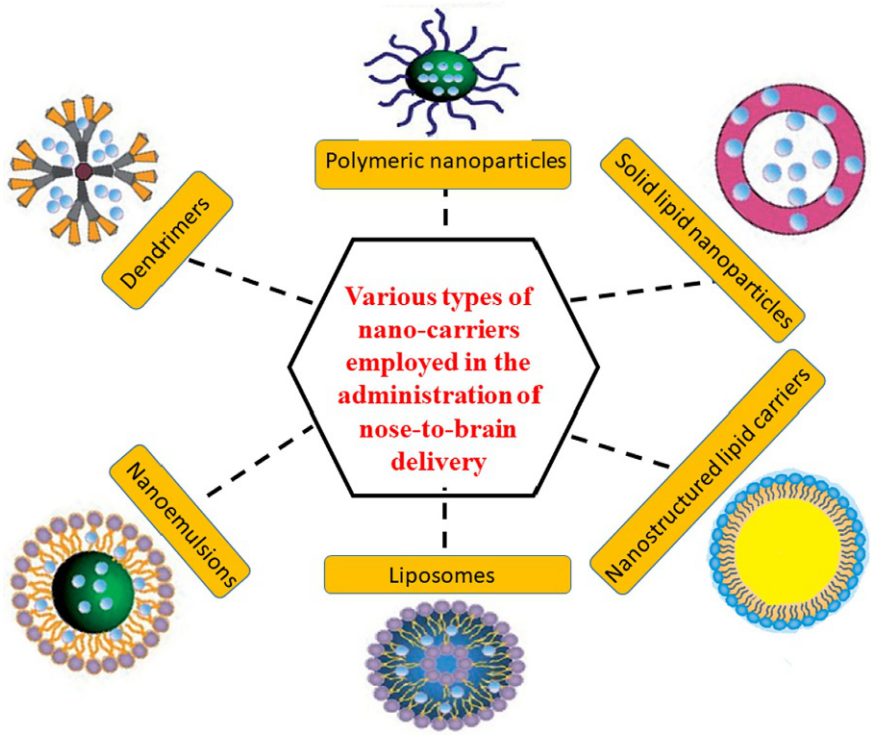

Fig. 2 Miscellaneous systems employed for the administration of nanomedicine via direct nose-to-brain passage.

Table 2 Nanocarrier formulations fabricated for nose to brain delivery in various neuronal disorders.

Nano-tool	Composition	Therapeutic agent	Average size (nm)	Remarks	Reference
Polymeric nanoparticles	NPs fabricated using ionic gelation of chitosan with tripolyphosphate anions	Bromocriptine	161.3 ± 4.70	Exhibited prominent surge in the dopamine content. Reversed akinesia and reduced catalepsy, enhanced brain uptake with targeting efficacy	[10]
	NPs of chitosan processed using ionic gelation	Bromocriptine	161.3 ± 4.70	Enhanced brain uptake	[85]
	Chitosan glutamate nanoparticles (RAS-CG-NPs) prepared using ionic gelation of CG with tripolyphosphate anions	Rasagiline	151.1 ± 10.31	Enhanced brain bioavailability	[86]
	Chitosan-based nanoparticles embedded with levodopa (CNL) blended with a thermo-reversible gel of Pluronic PF127 (CNLP gel)	Levodopa	<200	Enhanced residence time leading to better brain uptake, avoidance of levodopa degradation in peripheral circulation	[87]
	Poly(ethylene glycol)-poly (lactic-co-glycolic acid) (PEG-PLGA)-based nanoparticles	Odorranalectin	114.8 ± 5.60	Enhanced brain uptake therapeutic response in experimental PD	[88]
	Lectins altered polyethylene glycol-polylactide-polyglycolide (PEG-PLGA) nanoparticles	Fibroblast growth factor	104.8	Promoted the release of choline acetyltransferase	[89]
	Chitosan-based nanosuspension prepared using ionic-cross-linking technique	Donepezil	100–200	Nanoparticles formulation exhibited higher percentage of radioactivity per gram in the brain compared to donepezil solution	[85]

	Chitosan-based nanosuspension ionic-crosslinking method	Donepezil	150–200	No mortality, hematological alterations, body weight fluctuations, and toxicity in animals upon the delivery of nanosuspension in various doses	[90]
	PLGA nanoparticles (NPs) prepared using the solvent emulsification solvent diffusion technique	Donepezil	89.67 ± 6.43	Polysorbate 80-coated PLGA nanoparticles prominently enhanced permeation of donepezil to the brain as compared to the free drug solution	[91]
	Chitosan nanoparticles prepared using ionic gelation method	Rivastigmine	163.7 ± 7.6	Enhanced uptake into the brain and improved bioavailability	[92]
	Chitosan nanoparticles using ionic gelation of chitosan with tripolyphosphate anions	Estradiol	269.3 ± 31.6	Prolonged retention and enhanced brain uptake	[93]
NLCs and SLNs	NLCs prepared using microemulsion technique	Curcumin and Donepezil	<50	Higher uptake of both drugs in the brain leading to better memory and learning in mice. Elevated levels of acetylcholine in brain. Mitigated oxidative damage	[94]
	SLN prepared using stearylamine and Pluronic F-68	Ropinirole	98.43 ± 3.3	Diminished dosing frequency and better stability	[95]
	Polymer-lipid hybrid nanoparticles (PLN) prepared using emulsification solvent diffusion technique	Ropinirole	66.22 ± 6.2	Prolonged retention with limited detrimental effects on nasal cavity along with robust results in pharmacodynamics	[96]

Continued

Table 2 Nanocarrier formulations fabricated for nose to brain delivery in various neuronal disorders.—cont'd

Nano-tool	Composition	Therapeutic agent	Average size (nm)	Remarks	Reference
	Phospholipid-based gelatin nanoparticles	Fibroblast growth factor	143 ± 1.14	Elevated exogenous fibroblast in the olfactory bulb and the striatum with prominent therapeutic responses in a hemiparkinsonian rat model	[97]
Liposomes	Phosphatidylcholine, cholesterol and propylene glycol as edge activator	Galantamine	112 ± 8	Liposomes potentially blocked acetylcholinesterase following the delivery	[98]
	Liposome prepared using carrier material EPC, cholesterol, DSPE-PEG-CPP	Rivastigmine	–	Exhibited better ex vivo diffusion via goat nasal cavity as compared to rivastigmine solution	[99]
	1,2-distearyl-*sn*-glycero-3-phosphocholine (DSPC), cholesterol (CHE), polyethylene glycol (PEG).	Donepezil	102 ± 3.3	Prominent enhancement in donepezil concentration in brain compared to that of conventional formulation	[100]
	Niosomes were prepared using different nonionic surfactants namely span 20, span 60, span 80, tween 20, tween 80 and cholesterol using lipid layer hydration technique	Folic acid	3.05–5.625	Better absorption via nasal route as compared to drug solution used as control	[101]
Nanoemulsion	Lipidic nanoemulsion containing hyaluronic acid was prepared using Labrafac Lipophile and Labrafac PG as the oily phases, and tween 80 and Cremophor RH 40 as the surfactants	Curcumin and resveratol	115.2 ± 0.15	Therapeutically effective concentrations of both drugs entered the brain of the rat	[102]

	Nanoemulsion coated with chitosan was prepared using a blend of Capmul MCM, Captex 500 (oil phase) Tween 80, cremophor EL (surfactant), PEG 400 and Transcutol (cosurfactant)	Curcumin	14.4–118.9	Curcumin demonstrated enormous penetration in sheep nasal mucosa compared to those of placebo nanoemulsion and drug solution	[103]
	In situ ion-sensitive gelling system comprised of Capryol 90, Solutol HS15 and Transcutol HP	Curcumin	–	Targeting efficacy of in situ gelling system was found to be higher than plain drug solution	[104]
	Nanoemulsion prepared using Sefsol 218, tween 80, Transcutol and water	Ropinirole	58.61 ± 5.18	Enhanced penetration of ropinirole from nanoemulsions as compared to drug solution and improved pharmacodynamics performance	[105]
	Capmul MCM EP (−oil phase), Labrasol (surfactant), Transcutol-P as a cosurfactant	Rivastigmine	53.8–55.4	No sign of nasal ciliotoxicity	[106]
Dendrimers	Polyamidoamine (PAMAM) G5.NH_2	Paeonol	5.41 ± 0.24	PAMAM nanocomposite in situ gel significantly improved the nasal transport efficiency of nanocomposites	[107]
	e-PAM-R, a biodegradable PAMAM dendrimer	siRNA	188.7 ± 1.9	Intranasal delivery of siRNA offered an efficient means of gene knockdown-mediated therapy in the ischemic brain	[108]
	PAMAM dendrimer (1,4-diaminobutane core, amine terminated, Gen 5.0)	Haloperidol	15.10 ± 5.4	Improved brain targeting after nasal administration, with 6.7 times lower doses haloperidol formulation administered via the intranasal route producing behavioral responses that were comparable to those induced by haloperidol formulations administered via an intraperitoneal injection	[109]

polymers namely natural biodegradable and biocompatible materials as well as synthetic polymers have been employed to fabricate NPs for nose-to-brain delivery using various preparation approaches based on the drug and polymer characteristics [115]. Synthetic polymers (polycaprolactones and polyacrylates) render specific advantages over natural polymers (gelatin, collagen, chitosan, alginate, and albumin) primarily owing to their suitability to undergo any modification in inducing a wide range of desirable properties [83]. Chitosan NPs have demonstrated a reduction in mucociliary clearance and capability to momentarily open the tight junctions present between cells and thereby boosting the drug penetration in the brain from the nasal mucosa by the paracellular pathway [116]. In addition, chitosan NPs were reported to possess smaller particle size, enormous permeability across the nasal mucosa, and the capability to embed various drug molecules [92, 117]. The capability of chitosan NP to promote the access of rivastigmine to the brain via intranasal delivery has been explored [92]. The authors reported the drug transport efficacy to be $355 \pm 13.52\%$ with direct transport of $71.80 \pm 6.71\%$ in comparison with other formulations used as a control indicating the potential of rivastigmine-loaded NPs as a novel treatment alternative for AD. Several other studies reported the capability of chitosan nanoparticles to boost the delivery of donepezil, thymoquinone, and estradiol to the brain using the nose-to-brain approach [85, 93, 118]. The surge in the drug targeting and bioavailability in the brain of chitosan NPs was attributed to their better binding and accumulation in the nasal mucosa. Another study documented a promising diminution in nanoparticle interaction with opsonins following the coating with polysaccharide polymers [119]. Polyethylene glycol-poly caprolactone nanoparticles attached with ligand lactoferrin (LF) were reported as a robust nose-to-brain delivery system for the management of AD [120]. Chitosan NPs incorporated with rivastigmine and coated with polysorbate-80 depicted approximately 4 times increase in the rivastigmine levels in the mouse brain compared to the conventional intravenous administration [121]. Chitosan NPs loaded with galantamine hydrobromid were precisely penetrated to the brain within no time following its intranasal administration [122, 123]. Being economical compared to other biodegradable polymers, chitosan has been recommended to employ in the fabrication of polymeric NPs intended for the therapeutic intervention of neurotherapeutic agents [123]. Chitosan nanoparticles (CS-NP) were fabricated and evaluated in the brain delivery of bromocriptine (BRC) in mice with haloperidol-induced Parkinsonism following its nose-to-brain administration [124]. The authors reported a more promising reduction in akinesia and cataleptic behavior in mice receiving BRC-loaded CS-NP [124]. In another documented study, surface-engineered odorranalectin (OL) conjugated PEG-PLGA NP loaded with urocortin (UCN) were developed [88, 91]. These authors reported the brain delivery of UCN-loaded NP to be superior to that of acquired using drug alone.

8.2 Lipid nanoparticles

Lipid NPs are basically colloidal carriers, which may be alternatives to larger colloidal carriers such as polymeric NPs, liposomes, and nanoemulsions [125]. Lipid NPs may protect incorporated drugs against chemical degradation with specific capability of lipophilic drugs, controlled drug release characteristics, and limited bio-toxicology [126, 127]. In addition, lipid NPs can be produced easily at a moderate cost using ultrasonication techniques and high shear homogenization [128, 129]. Furthermore, NPs could enhance the penetration of drugs via the BBB via endocytosis owing to their lipophilic nature. Nonetheless, lipid NPs are also associated with some disadvantages for instance low incorporation of hydrophilic drugs, low in vivo stability, and the risk of surfactants and metal contamination upon the usage of ultrasound amid preparation [3, 127]. Lipid NPs consist of first-generation solid lipid nanoparticles (SLNs) and second-generation nanostructured lipid carriers (NLCs). The SLNs are basically solid lipid matrix in which the drug is either suspended or dissolved [130]. SLNs are generally perceived as comparatively stable colloidal carrier, involving the dispersion of a molten lipid in an aqueous surfactant by solvent emulsification-diffusion, high-pressure homogenization, solvent injection, and solvent emulsification-evaporation. SLNs are capable to diffuse through the tight junctions of the BBB endothelial cells [131] and also can evade the reticuloendothelial system (RES) and liver [131]. Furthermore, the SLNs can accommodate an elevated drug content compared to polymeric nanoparticles and thereby rendering controlled release profiles extending up to several weeks [131]. Biocompatible and physiologically well-tolerated triglycerides, fatty acids, or waxes approved for pharmaceutical application in humans are employed in the production of SLNs [132]. SLNs of rivastigmine were developed using the ultrasonication and homogenization method and evaluated. The authors reported an elevated ex vivo diffusion via goat nasal mucosa compared with rivastigmine conventional solution [133], which was ascribed to the lipophilic nature of the carrier. NLCs comprising of a blend of liquid lipids in solid matrix were developed to enhance long-term stability via polymorphic transformations of lipids to more stable forms with elevated drug loading [33]. The NLCs were reported to be readily assimilated after intranasal administration [60]. Drugs embedded in NLCs are usually well protected from degradation and efflux in the nasal cavity, which may potentially improvise their bioavailability both in the blood as well as in the brain. Polymer-lipid hybrid nanoparticles (PLN) loaded with ropinirole and SLNs embedded with ropinirole hydrochloride were fabricated for nose-to-brain administration via intranasal route. The emulsification-solvent diffusion method was employed for the preparation and the characterization was performed in terms of in vitro mucoadhesion, in vitro permeation, mucosal toxicity, and stability [95, 96]. The authors reported appropriate retention with negligible detriment on the nasal mucosa, along with suitable pharmacodynamic data [95, 96]. Gelatin nanostructured lipid carriers (GNLs) loaded with basic

fibroblast growth factor (bFGF) were developed using the emulsion and freeze-drying method [97] and were delivered via the nose-to-brain passage in a hemi-parkinsonian rat model. The GNLs exhibited and enhancement in exogenous concentration of bFGF both in striatum as well as in the olfactory bulb without any considerable damage to the mucous epithelium [97]. The authors suggested the suitability of GNLs as drug carriers via nose-to-brain route, particularly for bio-macromolecules [97]. Erythropoietin [134] and glial-derived neurotrophic factor (GDNF) [135] were adequately administered by adhering these protein therapeutics to mouse anti-TfR antibodies and authors reported promising neuroprotective responses.

8.3 Liposomes

Liposomes are spheroidal, coordinated bilayered, phospholipid sacs comprised of biocompatible biodegradable lipids similar to the lipids occur in human biologic membranes (cholesterol and phospholipids). Liposomes are capable of self-assembling into scaffolds consisting of an aqueous core and a lipid bilayer with sizes ranging between 50 nm and 100 μm. Liposomes can easily deliver both hydrophilic and lipophilic drugs because of their composition having both lipophilic as well hydrophilic segments [136]. Owing to their lipophilic nature, liposomes are considered as promising brain-targeting carrier systems [137, 138]. Liposomes are recognized as nontoxic and biocompatible carriers primarily owing to their phospholipid nature and can save the encapsulated drug molecules from enzymatic degradation and thus can boost their therapeutic efficacy [139]. Liposomes are fabricated using the film hydration methods (such as ethanol injection, ether injection, and hand shaking), heating and microfluidization, rendering high encapsulation efficiency [140]. The heating method is effectively scaled-up and is regarded as a secure method for the industrial-scale manufacturing of liposomes [141]. The pharmacokinetic profiles of the brain and plasma following the brain-to-nose delivery of a liposome formulation of donepizil in healthy male Wistar rats was compared [100]. The liposomes were reported to possess a spherical shape with smooth surface and maintained their integrity as a single unilamellar vesicle. Furthermore, liposomes did not show any aggregation with an average particle size of 102 ± 3.3 nm. A significant enhancement in donepezil content in the brain was found compared with that of the conventionally used products [100]. Elevated brain content of rivastigmine was measured following the nose-to-brain administration of cell-penetrating peptide (CPP) modified liposomes and rivastigmine-containing liposomes compared to the free drug [99]. Similar results were also reported in some other studies involving the rivastigmine-loaded liposomal formulations [142, 143], suggesting the potential of liposomes in the management of AD. In another study, nose-to-brain delivery of galantamine-loaded liposomes also emerged as a promising tool primarily because of successfully targeting the brain tissue [98]. These liposomes successfully inhibited brain acetylcholinesterase enzyme

shortly after nose-to-brain administration [98]. The average size of galantamine-loaded liposomes was 112 ± 8 nm with marvelous drug encapsulation efficiency, which predominantly ascribed to the intense hydrophilicity of the lipid bilayer and high lamellarity of the sac to the core [98].

8.4 Dendrimers

Dendrimers are nano-sized, radially symmetric molecules with well-defined, homogeneous, and monodisperse structure consisting of tree-like arms or branches [144]. These hyperbranched molecules were first discovered by Fritz Vogtle in 1978, by Donald Tomalia and coworkers in the early 1980s, and at the same time, but independently by George R. Newkome. The second group called synthesized macromolecules "arborols" means, in Latin, "trees." Dendrimers might also be called "cascade molecules," but this term is not as much established as "dendrimers" [145–147]. Dendrimers are nearly monodispersing macromolecules that contain symmetric branching units built around a small molecule or a linear polymer core [148–150]. "Dendrimer" is only an architectural motif and not a compound. Polyionic dendrimers do not have a persistent shape and may undergo changes in size, shape, and flexibility as a function of increasing generations [151–153]. Dendrimers are hyperbranched macromolecules with a carefully tailored architecture, the end groups (i.e., the groups reaching the outer periphery), which can be functionalized, thus modifying their physicochemical or biological properties [154–159]. Dendrimers have gained a broad range of applications in supramolecular chemistry, particularly in host-guest reactions and self-assembly processes. Dendrimers are characterized by special features that make them promising candidates for a lot of applications. Dendrimers are highly defined artificial macromolecules, which are characterized by a combination of a high number of functional groups and a compact molecular structure [160]. The emerging role of dendritic macromolecules for anticancer therapies and diagnostic imaging is remarkable. The advantages of these well-defined materials make them the newest class of macromolecular nanoscale delivery devices [161]. Dendritic macromolecules tend to linearly increase in diameter and adopt a more globular shape with increasing dendrimer generation. Therefore, dendrimers have become an ideal delivery vehicle candidate for explicit study of the effects of polymer size, charge, and composition on biologically relevant properties such as lipid bilayer interactions, cytotoxicity, internalization, blood plasma retention time, biodistribution, and filtration [162].

The structure of dendrimer molecules begins with a central atom or a group of atoms labeled as the core. From this central structure, the branches of other atoms called "dendrons" grow through a variety of chemical reactions. There continues to be a debate about the exact structure of dendrimers, in particular whether they are fully extended with maximum density at the surface or whether the end groups fold back into a densely

packed interior [163, 164]. Dendrimers can be prepared with a level of control not attainable with most linear polymers, leading to nearly monodisperse, globular macromolecules with a large number of peripheral groups as seen in Fig. 2, the structure of some dendrimer repeat units, for example, the 1,3-diphenylacetylene unit developed by Moore [165]. Dendrimers are a new class of polymeric belongings. Their chemistry is one of the most attractive and hastily growing areas of new chemistry [148, 166, 167]. Dendrimer chemistry, as other specialized research fields, has its own terms and abbreviations. Furthermore, a briefer structural nomenclature is applied to describe the different chemical events taking place at the dendrimer surface. Dendrigrafts are a class of dendritic polymers like dendrimers that can be constructed with a well-defined molecular structure, i.e., being monodisperse [168]. The unique structure of dendrimers provides special opportunities for host-guest chemistry (Fig. 2) and is especially well equipped to engage in multivalent interactions. At the same time, one of the first proposed applications of dendrimers was container compounds, wherein small substrates are bound within the internal voids of the dendrimer [169]. Experimental evidence for unimolecular micelle properties was established many years ago both in hyperbranched polymers [170] and dendrimers [171]. Brain-targeted drug delivery systems based on polyamidoamine (PAMAM) dendrimers with targeted modification have been successfully constructed more and more. Paeonol (PAE) was selected as a representative drug to study the drug loading and drug release ability of the PAMAM dendrimers-based drug delivery system [107]. Nasal administration of PAMAM-loaded small interfering RNA (siRNA) resulted in florescence-tagged siRNA being found in the cytoplasm and processes of neurons and of glial cells in many brain regions, including the hypothalamus, amygdala, cerebral cortex, and striatum [108]. PAMAM-loaded haloperidol improved brain targeting after nasal administration, with 6.7 times lower doses of the dendrimer-haloperidol formulation administered via the intranasal route producing behavioral responses that were comparable to those induced by haloperidol formulations administered via an intraperitoneal injection [109].

8.5 Nanoemulsions

Nanoemulsions are nano-sized emulsions with an average particle size smaller than 250 nm. Nanoemulsions can either be O/W emulsions or W/O emulsions and are transparent, clear, isotropic mixtures of water, oil, surfactant, and cosurfactant. Nanoemulsions are thermodynamically metastable but kinetically stable and their preparation requires intense energy and are manufactured with appropriate concentrations of surfactants [32, 136]. Nanoemulsions can mitigate the issues of drug delivery in terms of low solubility and bioavailability [3, 32]. There are two main techniques employed for the formulation of nanoemulsions, namely low-energy methods (ultrasound generators, e.g., spontaneous emulsification) and high-energy emulsification methods

(high-pressure homogenizers) [172, 173]. The type of oil employed amid the preparation of nanoemulsions can significantly influence their permeation into the brain. Fish oils and edible vegetative oil containing elevated content of polyunsaturated fatty acids (PUFA) such as omega-3 and omega-6-fatty acids can promote the brain uptake of drug-loaded nanoemulsions predominantly owing to identical structure to the natural fatty acids present in the brain [174–176]. Nevertheless, nanoemulsions have stability issues amid storage, phase separation, and burst release phenomenon. In spite of these demerits, numerous studies have reported the use of nanoemulsions to improve the drug delivery to the brain [103]. Curcumin incorporated into nanoemulsion was found to be safe as suggested by in vitro cytotoxicity and nasal cilio-toxicity studies. The droplet sizes and zeta potentials of curcumin nanoemulsions were reported in the ranges between 14.4 to 118.9 nm and −12.2 to −26.6 mV, respectively. The nanoemulsion of curcumin demonstrated higher influx across the nasal mucosa as compared to solution and plain nanoemulsions [103]. These results suggested the potential of nanoemulsions for the delivery of poorly soluble curcumin. In another study, hyaluronic acid-based lipidic nanoemulsion was reported to be a potential carrier for the delivery of curcumin and resveratrol for nose to brain [102]. The optimized hyaluronic acid-based nanoemulsion formulation showed a particle size of 115.2 ± 0.15 and a zeta potential of -23.9 ± 1.7. These authors showed significant concentrations of both drugs in the brain, with therapeutic levels being reached. Although some researchers found that nanoemulsions had the potential to deliver *Centella asiatica* plant extracts via the nose-to-brain route, they failed to measure the pharmacological response of the delivery system [177]. Intranasal delivery of chitosan-coated mucoadhesive nanoemulsions (o/w) loaded with ropinirole was investigated [105]. The optimized formulation showed a satisfactory globule size (58.61 ± 5.18 nm) and polydispersity index (0.201). Ropinirole levels were elevated in the brain, accompanied by a prominent enhancement in the AUC, half-life, and MRT; on the other hand, negligible amounts of ropinirole were found in liver, heart, spleen, lungs, and kidney [105]. This formulation showed improved delivery to the brain compared to that achieved using a ropinirole solution.

9. Conclusion

The BBB serves as an invincible barrier for a large number of drugs including anti-PD and anti-AD and anti-HD agents, and impede the development of therapies for neurodegenerative diseases. There is a need to develop novel therapeutic strategies to treat neurodegenerative disorders using noninvasive approaches and biodegradable agents that can bypass the BBB and deliver the pharmacological agents to specific sites in the brain. It is also necessary to prolong the release of drugs to ensure sufficient drug loading in the brain. Intranasal drug delivery to bypass the BBB for direct brain

targeting is increasingly gaining attention. Nanotechnology via the nose-to-brain route offers exciting possibilities and possesses the characteristics of an excellent delivery system. It is now well established that NP targeted to the brain should be very small, preferably between 100 and 200 nm. Nanocarriers such as polymeric nanoparticles, liposomes, and SLNs have shown satisfactory result in preclinical studies. However, the rapid entry into the brain of formulation components of nanocarrier systems such as polymers and preservatives following intranasal administration should be taken into account in addressing the safety of nanoformulations, this is not a concern following the systemic administration (IV, SC, IM, and oral) of nanoformulations because of the very extensive dilution in the blood stream. Consequently, to assure the safety of intranasal nanoformulations, their components must be simple and in low concentration. More toxicity, safety, and efficacy studies are required to confirm the preclinical findings to gain further support for clinical trials. Formulation strategies alone are insufficient to take advantage of this pathway for human intranasal drug delivery. Novel devices are being developed in an attempt to overcome the anatomical barriers of the nasal cavity and target formulation deposition in the olfactory region. Therefore, several issues such as the complexity of the brain, the mechanisms of drug transport via the intranasal route, and comprehensive neurotoxicological aspects need to be considered before nanoneurotherapeutics via the intranasal route become useful in clinical practice. In conclusion, the application of nano-enabled carriers via the intranasal route in the treatment of AD, PD, and HD could offer greater benefits if carrier surface is functionalized and new drugs are used.

Conflict of interest

The authors declare no conflict of interest.

Disclosures

There is no conflict of interest and disclosures associated with the chapter.

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CHAPTER 9

Oral drug delivery of nanomedicine

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1. Introduction

The purpose of drug delivery is to deliver the drug molecules to the site of action to get the desired therapeutic action [1]. The drug delivery is influenced by route of administration, commonly used administration routes are, parenteral (via injections), enteral (gastrointestinal tract), transdermal (via skin), inhalation, oral, and topical, etc. Among these, the oral administration route is the most preferred route due to its nature of being pain-free, high patient compliance, convenient to handle, noninvasive, and flexibility in dosage forms. Although oral administration is widely accepted drug delivery system, it faces numerous physiological and biochemical barriers such as the acidic and enzymatic environment in the gastrointestinal tract and various basement membrane barriers before reaching systemic circulation [2]. A detailed explanation of the biology and biological barriers of the oral drug is presented in the following subsections.

In oral drug delivery systems, the oral cavity serves as the primary port. Then it transits through the esophagus and reaches the stomach within 10 s [3]. In the stomach, the pH of gastric acid is 1.5–3.5 where most of the carbohydrates get digested [3]. This gastric pH enhances the decomposition of drugs [4]. The pH variation influences the dissolution of drugs which serves as a major limitation for the drug absorption [5]. The variation in pH ranging from 1 to 2 can lead to significant alterations in the drug dissolution and release kinetics. In Fig. 1, a detailed information has been provided regarding the pH and mucus average thickness at various sites of the gastrointestinal tract (GIT).

Apart from pH, the gastrointestinal microbiota and gastric emptying rate also play a crucial role in providing hindrance to drug absorption and degradation. The diversity of bacterial community inside human GIT, such as concentration gradually increasing toward jejunum, ileum, and colon hinders the orally administrated drug bioavailability.

Theory and Applications of Nonparenteral Nanomedicines
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After that, it comes in contact with the intestinal digestive environment consisting of various digestive and degrading enzymes such as lipases, pepsinogens, pepsins, bile, etc. which escalates the degradation and digestion process of the drugs [6]. Then the drug encounters with mucus barrier, which is the primary physical barrier present before the epithelium layers of GIT. The primary role of the mucus barrier is to protect the epithelial cells from foreign materials by the means of lubrication, binding of pathogen, and providing hindrances to these particles [7]. Mucus has a complex composition of carbohydrates, proteins, lipids, bacteria, antibodies, salt, cellular debris, etc. [8]. The Goblet cells are responsible for the intestinal mucus secretion. This mucus consists of mucins as their principal protein component which serves as a lubricant and aids the colonization of bacteria for providing protection against pathogens [9]. The protection of epithelium depends on the adherent mucus layer and Glycocalyx (carbohydrate-enriched coating) in the GIT [10]. Other functions that are shown by mucus layer apart from protecting the epithelial tissue are lubrication, colonization of good bacteria against the pathogenic bacteria, biological trapping of active molecules against inflammation, and healing process [11].

Fig. 1 provides information regarding the pH and the average mucus thickness in different segments (such as stomach, duodenum, jejunum, colon, and ileum) of the human

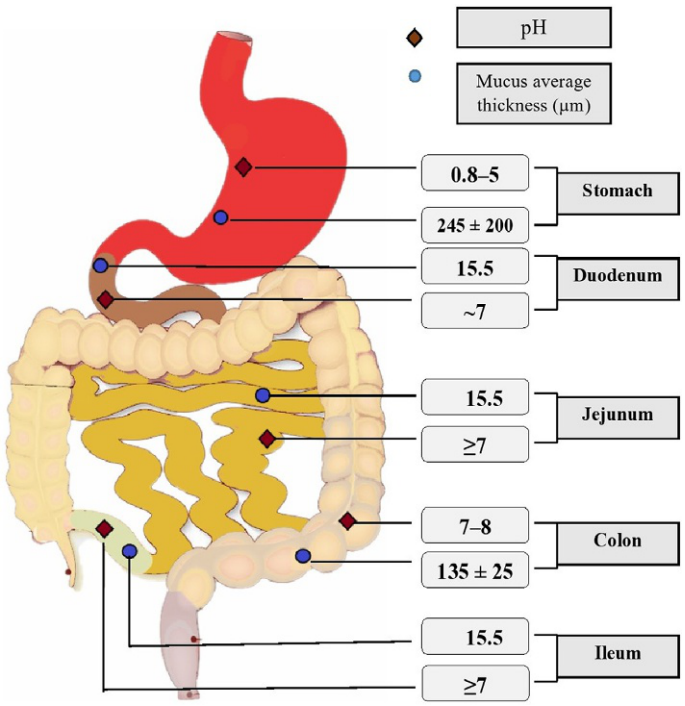

Fig. 1 pH and mucus average thickness values of different segments of the human GIT.

GIT. These mentioned pH and mucus average thickness serves as a major hindrance factor in oral drug delivery and the drug absorption [12]. The variation of pH and interaction with enzymatic environment provides difficulties in mucus permeation.

In addition, the presence of epithelial lining below the mucus barrier and various basement membranes serves as physical barriers before the drug absorption. These linings regulate the transport and transepithelial flux of macromolecules and ions from their apical to basolateral membranes. The adjacent intestinal cells restrict the passage of these molecules and ions. The tight junction protein complexes, adherens junctions, and desmosomes are involved in the restriction of molecular passage into the systemic circulation [1]. To transfer the drug across the epithelial layer there are mainly four types of delivery mechanisms such as transcellular, paracellular, carrier-mediated pathway, and facilitated transport pathways. In Fig. 2, a detailed schematic diagram of all the transport pathways across epithelial linings has provided. The transcellular pathway is the main mechanism for drug absorption which is the process of transporting the drug across epithelial cells by pinocytosis. However, the paracellular pathway is a mechanism of transport of drugs across the epithelial cells through the tight junction [12]. So the permeability through these impenetrable junctions of epithelial cells serves as the primary means of transportation of peptides and nanoparticles into the systemic circulation [14]. Some rare

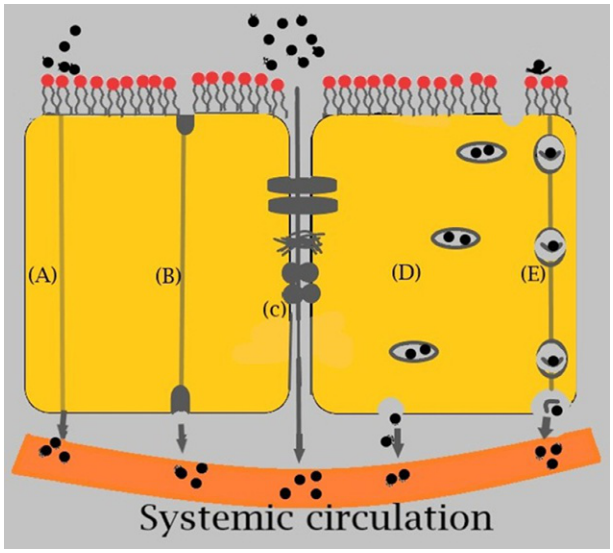

Fig. 2 This figure represents the transportation of drugs across the epithelial layers to get involved in the systemic circulation. Transport pathways have been represented: (A) transcellular absorption (primarily by lipid-soluble), (B) absorption of drugs through transport protein, (C) paracellular transport (primarily by water-soluble drugs), (D) pinocytosis, and (E) endocytosis of drugs by the means of a drug molecule [13].

epithelial cells like microfold cells or M-cells are given much attention due to their ability to show a comparatively very high rate of transcytosis which is very important for creating a delivery route [15–17].

Furthermore, bioavailability is one of the important pharmacokinetic properties of a drug, which determines its effectiveness in getting absorbed or reaching the systematic circulation. Parameters like solubility of drugs at the site of absorption and permeability through different biological barriers determine the bioavailability of the drugs. The degree of dissolution is an important parameter that determines the concentration of the drugs in the systemic circulation. The permeability factor of drugs determines its interaction with the molecules, membranes that come across during their delivery. The factors like slow dissolution, poor solubility in an aqueous medium, poor stability across pH variations, poor permeability affect the bioavailability of the orally delivered drugs. Various approaches that need to be undertaken to maintain the bioavailability of drugs are pharmaceutical approaches (modification of drug manufacturing processes and its formulation), alteration of different physiochemical characteristics (without altering the chemical structure), and pharmacokinetic approaches for chemical structure modification. All these approaches can lead to the enhancement of drug bioavailability [18].

The reduction of particle size will play a crucial role as it will enhance the surface area which will overcome various mechanical stresses and will increase the dissolution rate [19]. Other pharmaceutic approaches like crystal structure modification, dispersion of drugs in carriers, complexation, solubilization by surfactants, and chemical modifications can be utilized for the betterment [19]. The use of different kinds of solvents and polymeric states can lead to the alteration of crystal habit [20]. The drug dissolution can be enhanced by the complexation between more number of molecules. Such an effect will result in the formation of a nonbonded entity with well-defined stoichiometry essential for drug dissolution [14]. Different chemical modifications also required according to various sites of pH and other harsh environments for enhancement of the drug solubility. Apart from solubility and dissolution, the drug stability factor also plays a crucial role in the enhancement of bioavailability which is highly dependent on the choice of polymer coating and its thickness. These coatings delay the drug release event even after getting interacted with various harsh gastrointestinal environments. The drug delivery system should have the ability to disable the efflux pump (transport protein responsible for the exclusion of foreign/toxic/drug substances out of the cell) [21] so that the *p*-glycoprotein would not be able to pump out the drugs. Some necessary molecular compounds like vasopressin, corticotropin, and insulin have highly degradable properties if taken orally [16].

The bioavailability of the drugs via oral routes develops some limitations in terms of low stability, poor solubility, and poor permeability while coming in contact with some intestinal membranes and GIT. To perform efficiently, the drug delivery system should adopt various techniques to overcome difficulties like continuous mucosal secretion and acidic gastric environments [9].

The conventional drug delivery system failed to improve the bioavailability of less soluble and low permeable drugs and biologics; it lacks targeted delivery of drugs which leads to side effects and utilization of high doses. All these drawbacks can be eliminated by the use of nanotechnology-based methodologies for the oral delivery of drugs. So, the priority for a more efficient and protective delivery vehicle which can protect from various harsh environment became significant which leads to the preference of nanocarriers as efficient delivery vehicles [22].

2. Nanocarriers in oral drug delivery

Nanocarriers are nanomaterials that are small in size designed to carry the drug through GIT and to deliver the drug at the site of action [23]. They protect the drug from the harsh GIT environment and facilitate the delivery of the drug to the site of action (targeted drug delivery). In the nanotechnology-based oral drug delivery system, the drug is loaded to a nanomaterial by encapsulation or by complexation the resulting entity is called nanocarrier [23].

2.1 Properties of ideal nanocarriers for oral drug delivery

(1) Protection of the drug from harsh GIT environment, (2) enhances the absorption of the drug in the intestine, (3) accumulation inside specific cell in the body, (4) controlled release of the drug inside the specific cells [23], and (5) reduce immunogenicity

Nanocarriers that are used in oral drug delivery are classified into different types depending on the type of materials used. In this chapter, we explained about the most widely used nanocarrier systems such as polymeric nanocarriers and lipid-based nanocarriers.

2.1.1 Polymeric nanocarriers in oral drug delivery

Polymeric nanocarriers can be natural, synthetic, or combination of both. The selection of polymer-based nanomaterials for oral drug delivery should be based on biocompatibility, consistency, desirable interactions with drugs, physicochemical properties, drug release kinetics, and ease of chemical modification.

Naturally occurring polymers can be classified into proteins, polysaccharides, and polynucleotides. Natural polymers are highly biocompatible and biodegradable [24–26]. They are naturally abundant and modification is easy to enhance their therapeutic effect. But natural polymers have some disadvantages, such as uncontrolled water uptake, variation in the properties depending on source, contamination with microbes, unpredictable degradation pattern, and poor mechanical strength. To overcome these disadvantages chemically modified natural polymers are being used, for example, derivatives of chitin, dextran, alginate [27], etc.

Chitin is a biopolymer mostly found in the cell walls of invertebrates and fungi. It is a polymer of *N*-acetylglucosamine [28]. Chitosan is produced from chitin by partial alkaline deacetylation. So, chitosan is a copolymer of *N*-acetyleglucosamine and D-glucosamine [24, 29]. It is an excellent FDA approved biopolymer with desirable properties like biocompatibility, nontoxicity, biodegradability, mucus adhesion, antimicrobial properties, and its easy modification due to its reactive functional groups [30–34]. It is cationic and hydrophilic in nature and shows good mucoadhesion and can transiently open tight junctions between epithelial cells to enhance drug absorption [29, 34–36].

3. Nanocarrier-based drug delivery approaches

The nanocarrier systems face three major biological barriers before getting absorbed into systemic circulation such as,

1. Movement of nanocarriers across intestinal mucus barrier.
2. Movement of nanocarriers across intestinal epithelial layer.
3. Movement of nanocarriers across the basement membrane.

All these three barriers can be addressed in the following subsections.

3.1 Movement of nanocarriers across intestinal mucus barrier

In order to deal with the mucus barrier, the nanocarrier systems follow two types of methodologies, that is, mucoadhesive and mucus penetrating. Furthermore, the mucoadhesive systems can be categorized into four broad systems like polymeric mucoadhesive system, lipid-based mucoadhesive system, optimized mucoadhesive system, and targeted mucoadhesive system. All these characterizations are well presented in Fig. 3. As the mucosal environment provides harsh difficulties for drugs to reside in

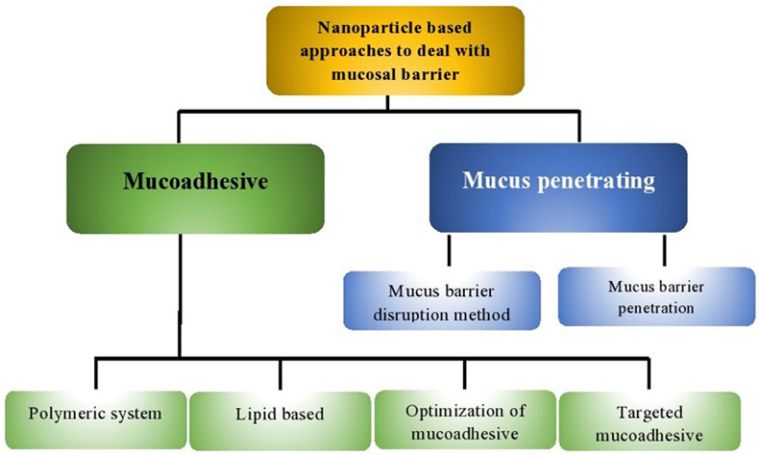

Fig. 3 Nanoparticle-based drug delivery approaches to deal with mucosal barrier.

the GI tract, method like mucoadhesion and mucus penetration systems are suggested as a solution for this to enhance the residing period. The orally delivered drugs by means of polymeric nanoparticles came out as one of the most significant methods to deal with the mucosal barrier. Polymers such as poly(sebacic acid) (PSA), poly(lactic acid) (PLA), poly(acrylic acid) (PAA), and poly(lactic-*co*-glycolic acid) (PLGA) are commonly preferred for the synthesis of mucoadhesive polymer systems [37]. Polymeric nanoparticles provide protection against proteolytic enzymes resulting in delayed residence time and enhanced bioavailability [38, 39]. Various experimental evidence also suggested direct uptake of Nanoparticles by the intestinal cells [40].

Lipid-based nanoparticles are important because they improve the solubility of hydrophobic drugs leading to improved bioavailability. Some lipid-based Nanoparticles are mixed micelles, liposomes, nanoemulsions, and solid-lipid nanoparticles [41]. It provides the advantage of solubility and increases in the bioavailability of hydrophobic drugs [42].

Optimization of the mucoadhesive property of nanoparticles by altering hydrophobicity/hydrophilicity, surface charge, and size can lead to desired results [43]. Particles composed of hydrophobic components show a 100-fold fast absorption than the hydrophilic cellulose polymer made nanoparticles [44]. Surface charge optimization of nanoparticles also influences the drug absorption characteristics, as it was proved by chitosan-based polymeric nanoparticles [45]. The size of polymeric nanoparticles also plays a pivotal role in the efficient uptake of drugs. Many studies indicated that mucoadhesive drugs are more efficient in terms of drug absorption as compared to nonmucoadhesive drugs [46, 47].

The targeted mucoadhesive approach generally comprises of coating of the nanoparticle with specific ligands that specifically bind to certain cells of the intestine (M-cells and goblet cell) which enhances the binding specificity resulting in the particle uptake [9, 48]. Furthermore, the mucus penetrating systems are broadly classified into mucus barrier disrupting and mucus barrier penetrating nanoparticle. As discussed by Ensign, disruption of the mucosal barrier cannot be compromised to improve the movement of nanoparticles across the mucosal barrier, because it may lead to the inclusion of foreign particles and pathogens into systemic circulation through the disrupted mucus [48, 49]. So, nanoparticles with reversible mucus penetrating properties are preferable [50].

3.2 Movement of nanocarriers across intestinal epithelial layer

The nanocarrier systems follow various pathways to surpass the complex obstacles before getting absorbed by systemic circulations. As the intestinal epithelial barrier remains as the primary obstacle for oral drug delivery, several methods have been addressed for overcoming these difficulties. Fig. 4 represents all the probable pathways including endocytosis, transcytosis, M-cell absorption, passive transcellular absorption, persorption, and

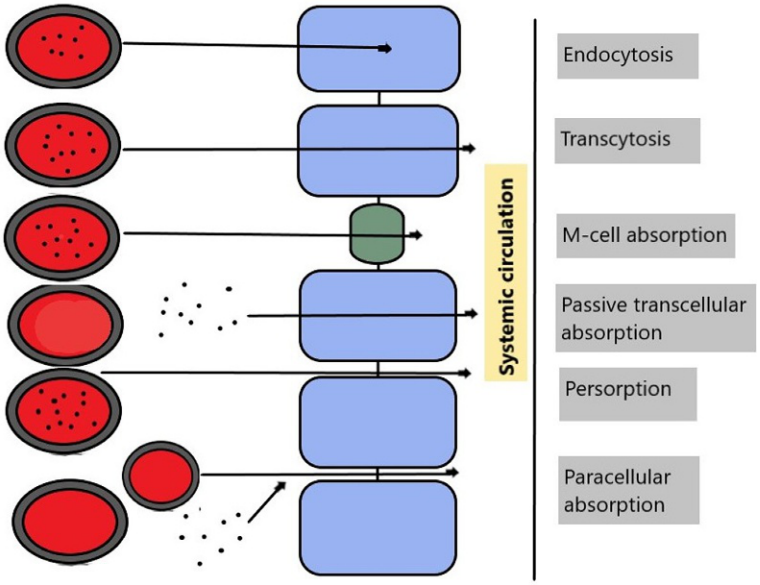

Fig. 4 Representation of advanced routes of transportation of nanocarrier systems into the systemic circulation through epithelial cells.

paracellular absorption that a nanocarrier can undertake. The transcellular and paracellular pathways enable the nanocarrier systems to release their payload into the systemic circulation. Apart from that, they can use the M-cells to reach lymphoid systems [51–53].

Various studies have been discussed in the following subsection regarding the interaction between the nanocarrier systems and epithelial layer.

Delivery of nanoparticles via the transferrin transport pathway evolved as a potential mechanism. Transferrin as a potential ligand facilitates the feasibility in targeting and delivery of drugs across biological barriers especially the blood-brain barrier [54]. The GIT comprises of transferrin receptors (TFR) [55]. Despite promising applications in certain aspects such as the TFR distribution over the basolateral surface of polarized cells, it faces significant hurdles [56].

In the case of oral drug delivery of biologics, the TFR-mediated transcytosis suggested the approach of TF recombinant fusion protein [57]. In some works based on nanomedicine, biologics delivery suggested the transepithelial trafficking of vitamin B12, IgG, and folate performed by adsorption of nanoparticles with the fragment crystallizable (Fc) region of vitamin B12 (airway and intestinal pathway) [58], IgG (at airway epithelium) [59], and folate (airway epithelium) [60]. The work of Jing M suggests enhanced uptake speed of nanocarriers and their translocation across monolayers analogs to the intestinal model [61].

A study on orally administrable nano-formulation for zika virus suggested the synthesis of optimized synthetic nano-formulation of drug ivermectin (IVM) that can create a passage into the bloodstream by overcoming the epithelial barrier [62].

The efflux transport system present in brain capillary enables the removal of undesirable substances results in increasing the complicacy for the cure of CNS-based disorders. Various studies widely suggested the use of nanocarriers for blocking the vesicular trafficking mechanism of the epithelial cells of brain capillary [63]. Then the nanoparticles can be directed toward specific parts or tumors such as glioblastomas [64] and protein aggregates like senile plaques [65].

It is well known that the presence of intercellular tight junction complex in intestinal epithelial cells prevents the delivery of macromolecules. Studies have shown the use of anionic nanoparticles for inducing tight junction relaxation which enhances the intestinal permeability. The Nanoparticle properties like size and surface charge decide the permeation-enhancing effects [66]. The importance of nanoparticle charge as compared to the size has also been shown for determining its interaction with intestinal epithelial cells. The study has also signified higher cellular uptake levels and toxicity due to positive nanoparticles as compared with the negative ones. But the negative nanoparticles showcased an efficient transport effect across the Caco-2 model which resembles the intestinal epithelial surface [67].

3.3 Movement of nanocarriers across basement membrane

Apart from mucosal and epithelial barriers, the barrier effect caused by basement membranes also provides difficulty in the movement of nanoparticles. Nanoparticles need to have a nanometer size to get pass through the minute pores of basement membranes. Most importantly its surface properties will decide its diffusion ratio across basement membranes. Studies based on nanoparticles diffusion across ECM (a 3D form of basement membrane) indicate its characteristic properties for disallowing the positively and negatively charged particles through it [66]. It was suggested that the exhibition of electrostatic interactions between nanoparticles and the components of ECM might be responsible for inhibiting the diffusion of nanoparticles [67].

Oral drug delivery of biologics has also been established as one of the potential methods for dealing with various diseases. However, oral drug delivery methodologies faced many biological obstacles during its delivery to the targeted site. Protein and peptide-based drugs (Biotherapeutics) faces difficulties when delivered orally. They undergo degradation before reaching the absorption site which decreases their bioavailability. To improve the oral bioavailability of these biopharmaceutics, nanocarrier system is a good alternative. Some of the strategies to enhance the oral bioavailability of biopharmaceutics include, enhancement of drug contact time with mucus [68], compounds (such as chitosan) responsible for promoting the transient opening of the epithelial

[69, 70], introduction of surfactants for enhancing membrane fluidity and disruption of membranes [71, 72], proteolytic enzyme inhibitors for improving drug stability in gut-lumen [73, 74], improving the receptor-mediated transcytosis pathways, use of cyclodextrins for dissociation of protein aggregates [75], and the disintegration of mucus layer [76, 77]. A proof-of-concept study based on oral insulin delivery suggested a nanocarrier consisting of insulin and trimethyl chitosan (TMC) composed core and a mucus-inert hydrophilic coating of *N*-(2-hydroxypropyl) (a derivative of methacrylamide copolymer pHPMA). These nanocarriers penetrated the mucus by an illustration of free Brownian motion resulting in the delivery of the core into the epithelial cell surfaces [78].

4. Applications of nanocarriers in oral drug delivery

The size and surface properties of the nanocarriers determine the efficacy of the oral drug delivery system. The pore size of the mucous layer approximately suggested to be around 0.2 μm, hence the preference of nanomedicines is widely suggested [79]. The surface property of the nanocarriers decides the permeability effect by reducing the mucosal behavior which enhances the absorption effect of the drugs at epithelium tissue. Various studies suggested that most of the synthetic nanocarriers are immobilized by mucus layer resulting in entrapped drug in mucus [11, 80]. The surface decoration of nanocarriers by polyethylene glycol (PEG) came out to be one of the effective strategies for enhancing the mobilization of nanocarriers through mucus layers. The permeability factor across mucus increased by 3- to 10-folds than the unaltered ones [81, 82].

The nanotechnology-based approaches evolved as a boon to chemotherapy in cancers, diabetes, psychiatric, etc. In the case of cancer treatment, the nanotechnology-based chemotherapeutics and imaging agents emerged as leading cancer nanomedicines which enhanced the pharmacokinetics, drug targeting, and payload release in a more efficient way [83]. The traditional cancer therapeutic actions faced lots of limitations including drug stability and solubility, poor bioavailability, chemoresistance, etc. The nanotechnology-based approaches addressed all these limitations by enhancing the methodologies of multiple targeting levels, smart delivery, and extended-release drug delivery systems [84]. Advancements in oral chemotherapy techniques will improvise the patients' quality of life.

Apart from nanomedicine-based oral chemotherapies, the nanomedicine-based oral insulin delivery also evolved as one of the primary technological advancements in the therapeutic management of type I and type II diabetes. Oral insulin administration becomes preferable over injections. Various nanocarriers based on polymeric, lipid, alginate, dextran compounds are widely preferable for oral insulin delivery in a sustained manner [85].

For psychiatric illnesses like schizophrenia, anxiety, and depression the oral dose for pharmacological treatment options are highly preferable. With nanomedicine-based

technological advancements, oral administrations can behave efficiently by the use of targeted nanocarriers. Various studies have been done regarding the challenges of psychiatric drugs. Currently, research is focused on designing nanocarriers based on nanoemulsions, dendrimers, solid lipid nanoparticles, polymeric micelles, and different biodegradable polymers [86]. A detailed emphasis has given in the next subsection of our chapter regarding the application of these nanocarriers in oral drug delivery.

Liu et al. demonstrated that the surface nature of the nanoparticles (hydrophilic/hydrophobic) is important in drug delivery. They showed the effect of hydrophilic and hydrophobic surface nature of chitosan nanoparticles on the insulin bioavailability in diabetes mellitus rats. Diabetes mellitus is a physiological disorder in which our system cannot control the blood glucose level due to insufficient or lack of production of insulin [87]. People suffering from diabetes mellitus must take insulin from outside on a daily basis [88]. Till now hypodermal injection is the way to administer insulin. Oral administration of insulin is not successful because insulin will undergo degradation due to acidic milieu of the stomach and enzymatic environment of the intestine [89, 90]. So oral bioavailability of insulin is relatively low [91]. Nanocarriers are under investigation to improve the oral bioavailability of insulin. To enhance the oral bioavailability of insulin, nanocarrier has to protect insulin from degradation from the acidic milieu of the stomach, enzymes of intestine and it has to cross the mucus layer and epithelial layer of the intestine to deliver the insulin into the blood. To deliver the insulin into the blood, nanocarriers should have mucoadhesive and mucus penetrating property [91]. Mucoadhesive property increases the retention time of the nanocarrier in the GIT so that it enhances in vivo absorption [33]. Mucus penetrating property facilitates the penetration of the nanocarriers through the mucus layer of the intestine and to reach the epithelial layer. Once they reach the epithelial layer, they can enter blood either by transcytosis or by paracellular pathway [33]. These mucoadhesive and mucus penetrating properties are governed by the surface nature of the nanocarriers. They modified the hydrophilic nature of chitosan nanoparticles by grafting with different ratios (5%, 10%, and 18%) of polyethylene glycol monomethyl ether (mPEG) and they tested the insulin delivery capacity of these nanocomplexes in rats. CS-mPEG$^{10\%}$-insulin nanocarriers showed least mucus adhesion property than CS-insulin, CS-mPEG$^{5\%}$-insulin, and CS-mPEG$^{18\%}$ nanocarriers. Then, they reduced the hydrophilic nature of CS-mPEG$^{10\%}$-insulin by adding glyceryl monocaprylate converting them into hydrophobic. They found that the absorption of insulin was high (5.2%) with 10% mPEG grafted chitosan nanoparticles (CS-mPEG$^{10\%}$). Their study indicated that, to improve the absorption of insulin by nanocarrier based oral drug delivery, the nanocarrier should have optimum hydrophilicity and lower mucoadhesion property [33] (Fig. 5).

Aqueous solubility of the drug is one of the important properties that determine the bioavailability of the drug in the body [92]. The other properties include dissolution rate, permeability of drug, first pass metabolism, susceptibility to efflux mechanisms, and

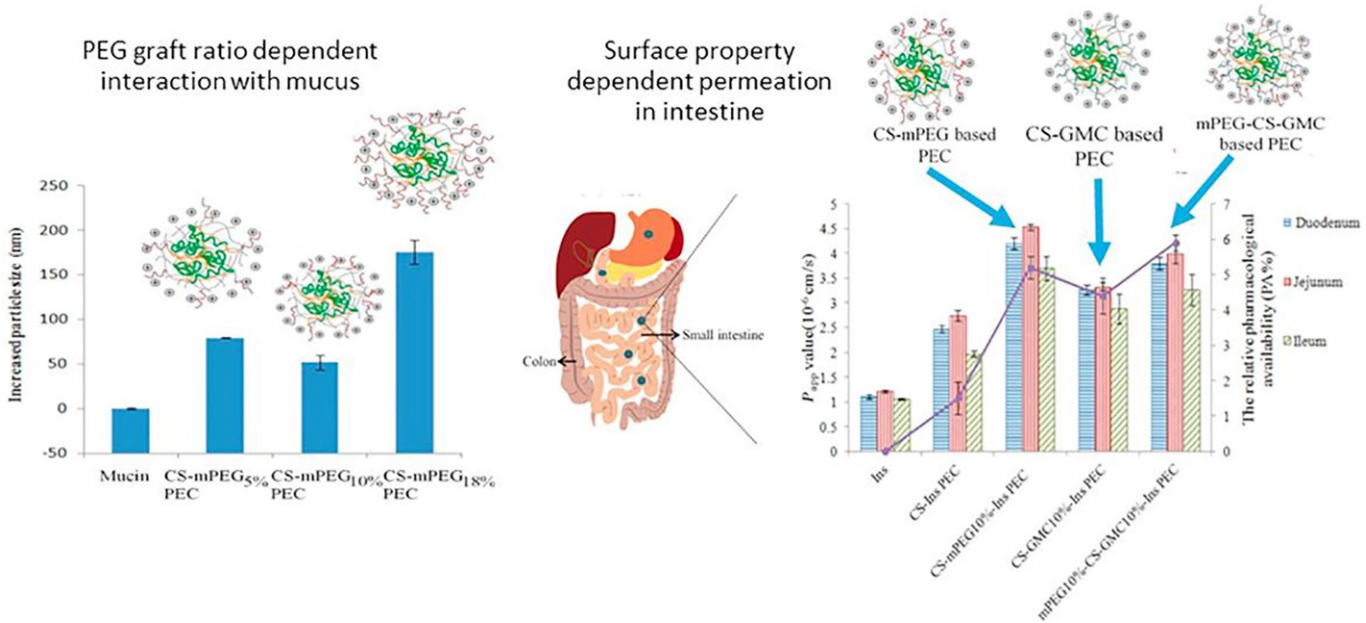

Fig. 5 Improved mucus penetration and bioavailability of insulin by CS-mPEG nanocarriers. *(Reproduced with permission from reference C. Liu, Y. Kou, X. Zhang, W. Dong, H. Cheng, S. Mao, Enhanced oral insulin delivery via surface hydrophilic modification of chitosan copolymer based self-assembly polyelectrolyte nanocomplex, Int. J. Pharm. 554 (2018) 36–47.)*

presystemic metabolism. Among these, the low aqueous solubility of the drug is the main reason for the low bioavailability when administered orally [93]. Most of the recently developed drugs have low aqueous solubility posing a challenge to the formulation scientists [93]. There are different approaches to improve the aqueous solubility of drugs. Some of them are—particle size reduction, changing the crystal habit like amorphous, polymorphs and cocrystallization, solid dispersions, change in pH, use of a buffer, derivatization, salt formation, etc. Nanomaterials can also be used to modify the solubility and hence the bioavailability of drugs [94]. For example, Lutein is a hydrophobic xanthophyll carotenoid found in leafy vegetables, corn, orange fruits, egg yolk, and spinach. It shows antioxidant, antidiabetic, antiobesity, anticancer, and antiinflammatory properties. Our body cannot synthesize Lutein so it was suggested to be taken through food. Despite its biological activities, it is sensitive to light, heat, pH, and oxidative stress. Even through food, it shows very low bioavailability (10%) because of its low aqueous solubility. Toragall et al. demonstrated in rats that the solubility and bioavailability of lutein (nutraceutical) can be improved by using modified chitosan nanocarrier that is chitosan-oleic acid-sodium alginate hybrid nanocarrier. They chose chitosan and alginate because they are highly biocompatible and biodegradable. Chitosan is a cationic copolymer of *N*-acetyleglucosamine and D-glucosamine. Alginate is an example for anionic polysaccharide. It is composed of guluronic acid and mannuronic acid. They prepared the nanocarrier by the combination of chitosan and alginate with a lipid-oleic acid and encapsulated lutein into them. The lipid core facilitates the binding of the lutein. The lipid core enhances the solubility of lutein. The polymeric surrounding (chitosan and alginate) protects lutein from the acidic and enzymatic environment of the GIT. The cationic surface of chitosan facilitates the binding of the nanoparticles to the mucus layer of the intestine. The biodegradable nature of the polymers helps in slow and sustained release of lutein. The biopolymer selection for the encapsulation of nutraceuticals depends on various properties of biopolymer, such as, density, charge distribution, type of ions on the surface, ionic strength, polydispersibility, molecular weight, concentration, pH, and inter and intramolecular forces. Selection of two or more biopolymers imparts synergistic effect in the improvement of functional properties like stability, solubility, bioavailability, and targeted delivery [95].

4.1 Dendrimers

Dendrimers are spherical polymers with high symmetry. They are characterized by (i) multifunctional core, (ii) repeated branches attached to the core, and (iii) multifunctional outer shell. Dendrimers are monodisperse polymers. Because of the high density of functional groups on dendrimers, they are water-soluble and they can be functionalized with different functional groups. They show high encapsulation efficiency. Commonly used dendrimers in oral drug delivery application are Polypropylene imine (PPI) and Polyamidoamine dendrimers [96, 97].

Cardiovascular disease is the main cause of death of over 17.1 million peoples a year. The main cause of cardiovascular diseases is Hypercholesterolemia which means the presence of high levels of cholesterol in the blood. Stanins (3-hydroxy-3-methylglutaryl

coenzyme A [HMG-CoA] reductase inhibitors) are the class of drugs used to control or decrease the cholesterol content in the blood. Stanins inhibit HMG-CoA reductase which catalyzes the reduction of HMG-CoA to mevalonate thus blocking the key step in the synthesis of cholesterol in the liver. Simvastanin is an example of stanin class of drugs. It belongs to BCS (Biopharmaceutics Classification System) class II. It suffers from poor aqueous solubility and hence poor oral bioavailability (<5%). Kulhari et al. demonstrated in a rat model that the oral bioavailability of simvastatin can be improved by encapsulating the drug in dendrimers. The purpose of choosing dendrimer-based nanocarriers was that they were proved to enhance the solubility of encapsulated drug [98]. Dendrimers have multiple functional groups on the surface which imparts its higher solubilizing property. Hydrophobic interior enables the encapsulation of hydrophobic drugs. Thus, dendrimers facilitate the higher aqueous solubility of drugs. Kulhari et al. used polyethylene glycol (PEG) attached polyamidoamine (PAMAM) dendrimers (G4-PAMAM-PEG) to test the oral bioavailability of simvastatin. They showed that G4-PAMAM-PEG nanoparticles exhibited highest drug encapsulation efficiency, the solubility of simvastatin was increased in the presence of dendrimers and the drug was released in a slow and sustained manner which means that efficacy of the drug (reducing the cholesterol content) was improved [97] (Fig. 6).

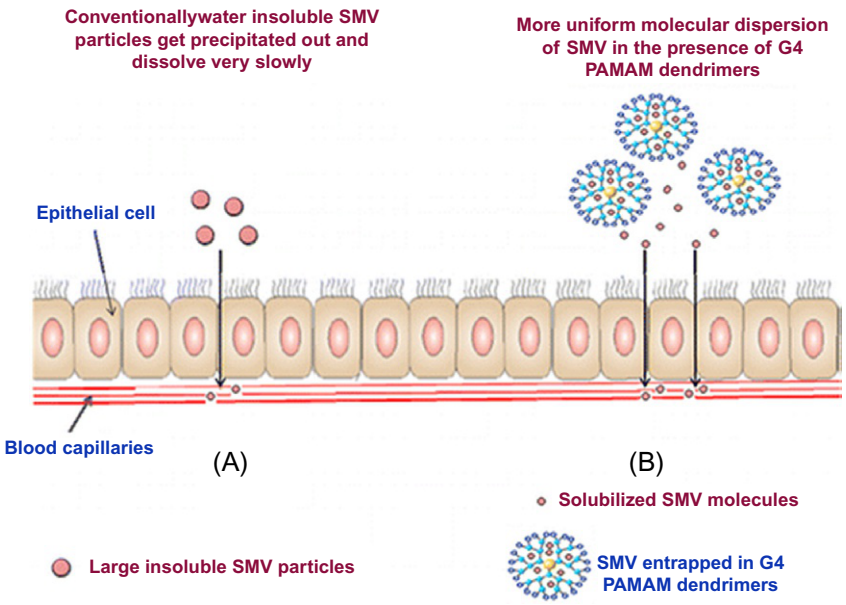

Fig. 6 Improved penetration and delivery of simvastatin by PAMAM dendrimer-based nanocarriers. *(Reproduced with permission from reference H. Kulhari, D.P. Kulhari, S.K. Prajapati, A.S. Chauhan, Pharmacokinetic and pharmacodynamic studies of poly(amidoamine) dendrimer based simvastatin oral formulations for the treatment of hypercholesterolemia, Mol. Pharm. 10 (7) (2013) 2528–2533.)*

4.2 Lipid-based formulations

Lipids are long-chain organic molecules. They are hydrophobic or amphiphilic. These lipid molecules can assemble together to form nano-sized particles which can be used in drug delivery applications. Mainly three types of lipid-based nanocarriers are in use. They are liposomes, solid lipid nanoparticles, and lipid emulsions.

Liposome is a spherical sac of phospholipid molecules enclosing an aqueous core. These are useful to carry hydrophilic drugs. Solid lipid nanoparticles contain a solid lipid core surrounded by surfactant layer. This lipid core is useful in carrying hydrophobic drugs. Lipid emulsions are mixtures of hydrophilic and hydrophobic components with the help of surfactants. These emulsions are mainly used for intravenous drug delivery.

Lipid-based nanocarriers are highly biocompatible, biodegradable, highly stable, exhibits good drug targeting, drug loading capacity is high, nontoxic, facilitates controlled drug release [99].

Quercetin (QR) is a phytochemical which is proved to have antiangiogenic effect and induce the apoptosis in cancer cells [100, 101]. But it is poorly water soluble and shows very low gastrointestinal permeability and hence shows very low bioavailability (1% in humans) [102]. So, it is not being used as an oral medication. Nanocarriers provided a good alternative strategy in designing oral formulation for Quercetin. Nagarsenker et al. demonstrated that phospholipid-based cationic nanocarrier-Leciplex (Soybean lecithin) can be used to enhance the bioavailability of Quercetin in rats. They encapsulated the quercetin onto Leciplex and the resulting nanocarriers (QR-LeciPlex) size was less than 200 nm. Leciplex is a phospholipid with cationic surfactant. Leciplex is composed of Soybean lecithin, dimethyldidodecyl ammonium bromide—a cationic surfactant and diethylene glycol monoethyl ether (Transcutol HP) as a solvent. QR-LeciPlex showed good antiinflammatory and antitumorigenic activity compared to the Quercetin suspension [103]. The enhanced efficacy of Quercetin with Leciplex could be of many attributes. Cationic phospholipids (LeciPlex) are known to have good mucoadhesion property because of electrostatic attraction between cationic surface layer and anionic mucus layer of the intestine and they show good permeation ability which helps in the absorption of the drug associated with them. LeciPlex is capable of protecting the encapsulated drug from the acidic and enzymatic environment of the GIT which minimizes the presystemic metabolism of the drug, so that bioavailability of the drug was improved. Lipids are known to produce nanoparticles in the size range 20–500 nm. The particles of this size range are preferentially taken up by lymphatic circulation so lipids improve the lymphatic uptake of drugs. This lymphatic uptake circumvents the first-pass metabolism and hence increases the bioavailability. LexiPlex is also composed of lipids and form nanocarriers of size ~400 nm which enhances the lymphatic transport of encapsulated Quercetein. This leads to improved bioavailability [102]. Phospholipids or lecithin exhibit good biocompatibility and can interact with a wide range of drugs

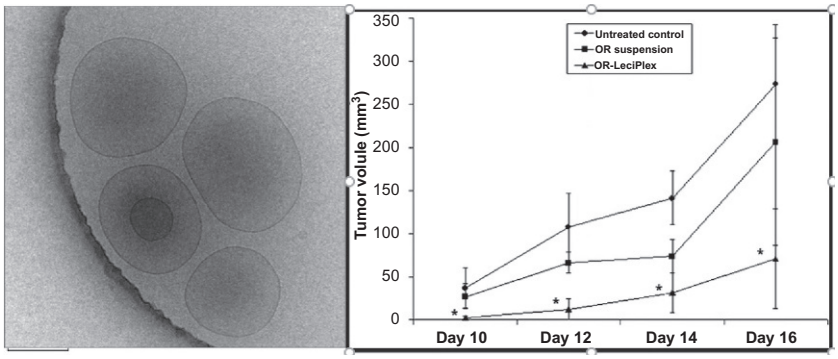

Fig. 7 Improved efficacy of Quercetin LeciPlex nanocarriers. *(Reproduced with permission from reference A.A. Date, M.S. Nagarsenker, S. Patere, V. Dhawan, R.P. Gude, P.A. Hassan, V. Aswal, F. Steiniger, J. Thamm, A. Fahr, Lecithin-based novel cationic nanocarriers (Leciplex) II: improving therapeutic efficacy of quercetin on oral administration, Mol. Pharm. 8 (3) (2011) 716–726.)*

(hydrophobic, hydrophilic, and amphiphilic). Solid-lipid nanoparticles, microemulsions, and liposomes are examples for phospholipid based nanocarriers. Cationic phospholipid-based nanocarriers are very important in designing oral drug delivery systems because they exhibit greater penetration and uptake across the GIT compared to anionic phospholipid-based nanocarriers [103] (Fig. 7).

4.3 Targeted delivery

Targeted drug delivery is a type of drug delivery system in which the medication is transported or concentrated selectively at the site of action to localize the interaction of drug with diseased site and to avoid the harmful effects to healthy tissue due to drug interactions. Chemotherapeutic agents used in cancer treatment are cytotoxic and nonselective that means they can kill healthy cells along with cancerous cells. If we can deliver these chemotherapeutic agents to only cancerous tissue then we can reduce the unwanted hazardous side effects of these agents. Advantages of targeted drug delivery systems are—we can reduce the frequency of dosage of the drug taken by a patient, can achieve the uniform effect of the drug, can reduce the side effects of the drug, and can reduce the fluctuations in circulating drug levels [104].

Cabazitaxel is a US FDA approved drug for prostate cancer. Its oral bioavailability is very less (around 20%). Tang et al. improved the bioavailability of the drug to 32.1% in rats by using nanocarriers. They encapsulated the drug onto polymer-lipid hybrid [poly (ε-caprolactone), medium-chain triglyceride and soybean lecithin] nanoparticles and these nanocarriers were loaded onto porous and hollow yeast cell wall microparticles. Thus, they formed nano-in-micro carriers. The reason for entrapping cabazitaxel loaded nanocarriers in the yeast cell wall is the yeast cells are porous microspheres with hollow cavities which can hold chemicals and small particles efficiently and the cell wall is

composed of β-1,3-D-glucan which can be recognized by apical cell membrane receptors of phagocytes. Dectin-1 found on the surface of phagocytic cells like macrophages, dendritic cells and specifically M cells of the intestine is the primary receptor of β-1,3-D-glucan. β-1,3-D-Glucan containing yeast cell microparticle with drug-loaded nanoparticles are easily taken up by M cells of the intestine by dectin-1-mediated endocytosis and then transported by macrophages to the circulation via the lymphatic system. Thus, yeast cells facilitate the selective uptake of the nano in microparticles with cabazitaxel in the intestine and nanocarriers facilitate the higher aqueous solubility of the drug which leads to high oral bioavailability. These nano-in-micro carriers showed sustained drug release and high stability [105] (Fig. 8).

Yuan et al. demonstrated that stearic acid-*g*-chitosan polymeric micelles increased the bioavailability of doxorubicin, a chemotherapy medication to treat cancer. Polymeric micelles have hydrophobic interior and hydrophilic exterior. The reason for choosing polymeric micelles (stearic acid-*g*-chitosan) is, polymeric micelles are more stable than surfactant-based micelles and critical micellar concentration of polymeric micelles is low compared to surfactant-based micelles imparting more stability to polymeric

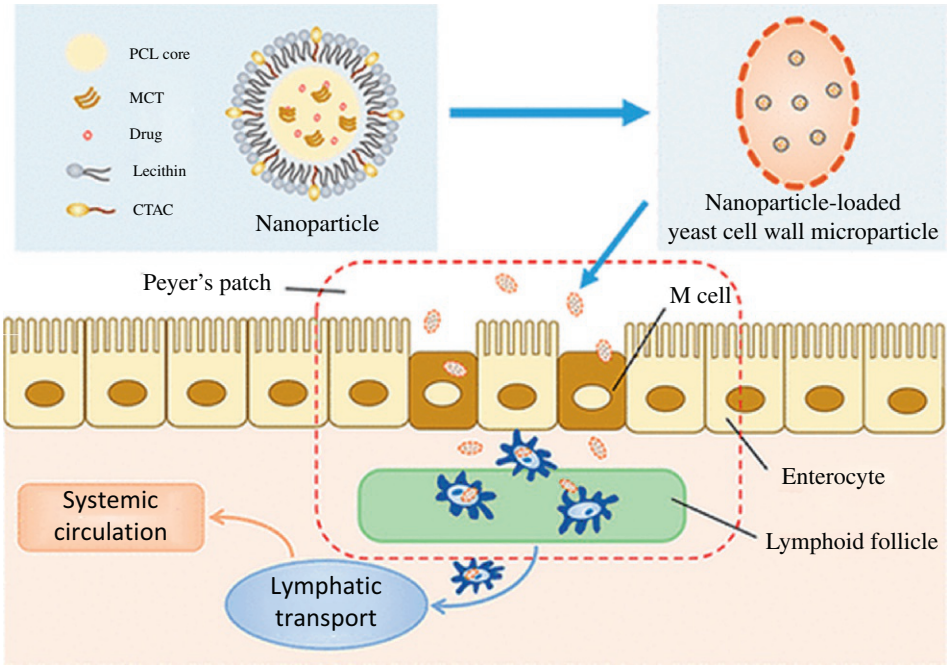

Fig. 8 Delivery of carbazitaxel in GIT by nano-in-micro carriers. *(Reproduced with permission from reference T. Ren, J. Gou, W. Sun, X. Tao, X. Tan, P. Wang, Y. Zhang, H He, T. Yin, X. Tang, Entrapping of nanoparticles in yeast cell wall microparticles for macrophage-targeted oral delivery of cabazitaxel, Mol. Pharm. 15 (7) (2018) 2870–2882.)*

micelles. Hydrophilic exterior facilitates the stability of the micelle in an aqueous environment, while the hydrophobic core facilitates the loading of hydrophobic drugs. Hydrophilic exterior protects the micelle from the gastrointestinal environment and provides anchoring of the micelle to the GIT. Stearic acid-*g*-chitosan-based polymeric micelles loaded with doxorubicin, with different amino-substituted degrees showed good permeability and bioavailability. This improved permeability and bioavailability is because of increased pH and concentration-dependent transcytosis process and also improved the paracellular transport pathway [106] (Fig. 9).

Amphotericin B (AmB) is an excellent drug used to treat systemic fungal infection and leishmaniasis. But it suffers from poor solubility and poor permeability and instability in the acid biological milieu. It causes hemolytic toxicity and nephrotoxicity when aggregated in the body. Because of these properties of the drug scientists are struggling to develop an oral formulation. Sanyogjain et al. demonstrated that by using polymeric-lipid hybrid nanocarriers the solubility and permeability can be improved and toxicity of the AmB can be reduced. They used gelatin-coated lipid hybrid nanoparticles to deliver AmB. They chose gelatin (cationic polymer) because it is highly biocompatible, biodegradable, low immunogenic, and low cost. They chose lecithin (anionic lipid) as lipid. Polymeric nanocarriers show more stability in biological fluids and lipid nanocarriers show increased gastrointestinal absorption and increased plasma concentration. In their simulated study, gelatin-coated lipid hybrid nanocarriers were loaded with AmB. AmB was protected from the acid environment by gelatin. Gelatin retarded the release time of

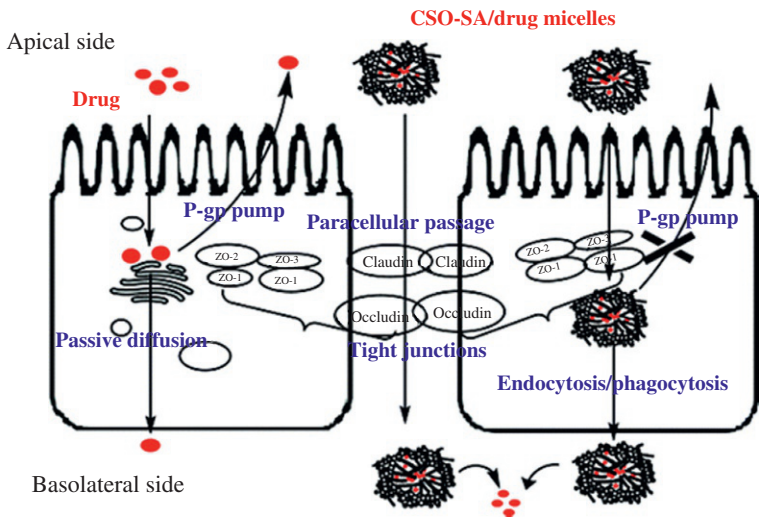

Fig. 9 Permeation and delivery of doxorubicin by stearic acid-*g*-chitosan nanoparticles. *(Reproduced with permission from reference H. Yuan, L.J. Lu, Y.Z. Du, F. Hu, Stearic acid-g-chitosan polymeric micelle for oral drug delivery: in vitro transport and in vivo absorption, Mol. Pharm. 8 (1) (2011) 225–238.)*

the AmB. Lecithin facilitated the absorption of the AmB. The toxicity of the drug also reduced by this hybrid nanocarrier drug delivery system [107].

Exenatide is a drug used for type 2 diabetes. It suffers from low bioavailability when administered orally because of degradation by the acidic gastric environment. Sun et al. demonstrated that, by encapsulating the exenatide in nanocarriers, the bioavailability of the drug can be enhanced. They prepared a block copolymer nanoparticle-CSKSSDYQC-dextran-poly(lactic-*co*-glycolic acid) (CSK-DEX-PLGA) and encapsulated the zinc salt of exenatide into it. CSK is cell-penetrating protein which has an affinity for goblet cells of the intestinal epithelium. This protein facilitates the adhesion and penetration of the intestinal epithelium. Dextran (DEX) and poly(lactic-*co*-glycolic acid) are biopolymers which are biocompatible and biodegradable. They provide protection to the drug and stability of nanoparticles. They prepared nanoparticles having CSK on the exterior and Dextran and PLGA forms the core of the particle. These nanoparticles were loaded with zinc salt of exenatide. Oral administration of these nanocarriers in type 2 diabetes mice has proved that exenatide-Zn^{2+}-CSK-DEX-PLGA nanoparticles gave stable control over blood glucose levels [108] (Fig. 10).

Oral administration of insulin is ideal because it is easy, safe, and more patient compliance. But oral administration of protein-based drugs like insulin has to face many

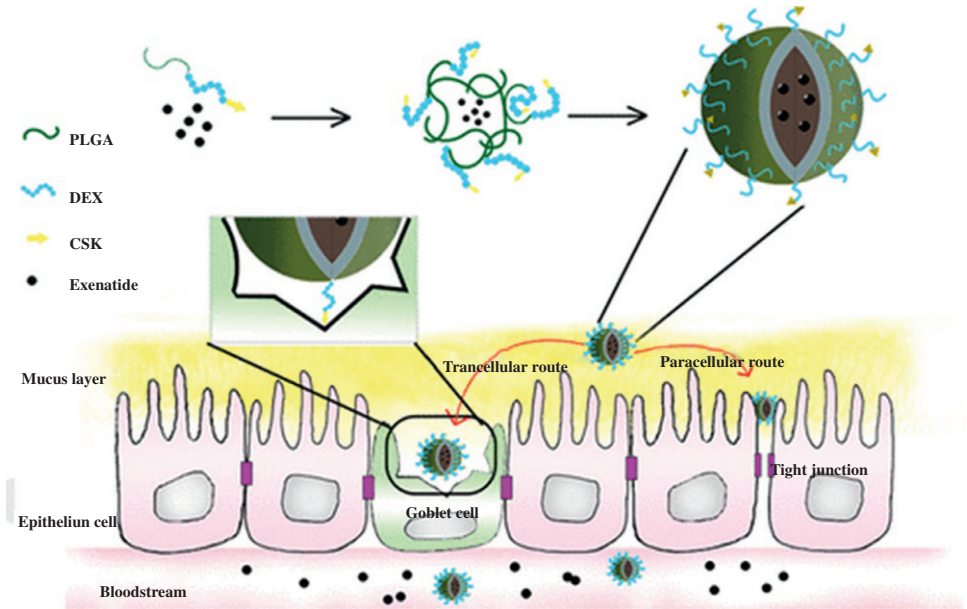

Fig. 10 Drug delivery through CSK-DEX-PLGA nanocarriers. *(Reproduced with permission from reference Y. Song, Y. Shi, L. Zhang, H. Hu, C. Zang, M. Yin, L. Chu, X. Yan, M. Zhao, X. Zhang, H. Mu, K. Sun, Synthesis of CSK-DEX-PLGA nanoparticles for the oral delivery of exenatide to improve its mucus penetration and intestinal absorption, Mol. Pharm. 16 (2) (2019) 518–532.)*

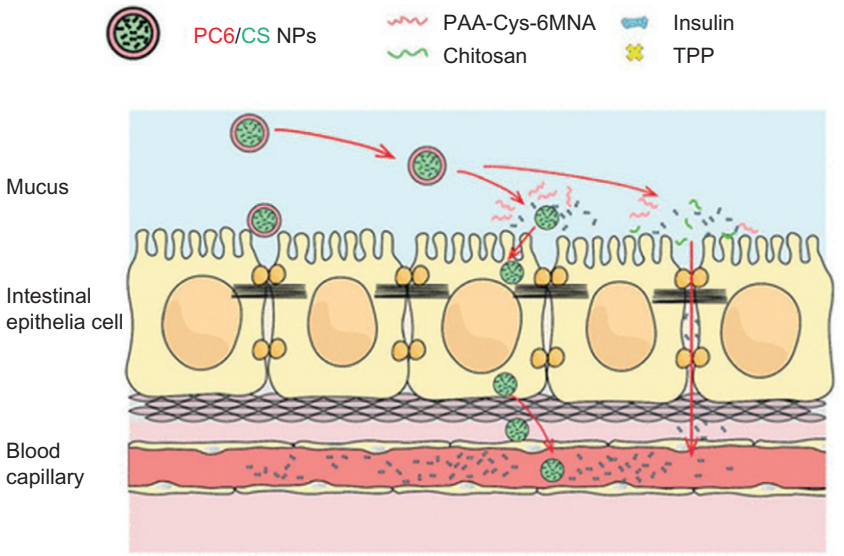

Fig. 11 Insulin delivery through PC6-coated chitosan nanocarriers. *(Reproduced with permission from reference S. Zhou, H. Deng, Y. Zhang, P. Wu, B. He, W. Dai, H. Zhang, Q. Zhang, R. Zhao, X. Wang, Thiolated nanoparticles overcome the mucus barrier and epithelial barrier for oral delivery of insulin, Mol. Pharm. 17 (1) (2020) 239–250.)*

biological barriers, such as acid milieu in stomach, proteases in the intestine, mucus layer of the intestine, and epithelial lining of the intestine. These barriers make the oral bioavailability of the protein-based drugs almost zero. Wang et al. improved the oral bioavailability of insulin to 16.2% in the diabetic rat model by using thiolated nanocarriers. They prepared chitosan (modified chitin) nanoparticles loaded with insulin and coated these nanoparticles with poly(acrylic acid)-cysteine-6-mercaptonicotinic acid (PC6) which is a type of preactivated thiolated polymer. This thiolated polymer (PC6) has the ability to interact with mucin in mucus layer by disulfide formation and facilitates the penetration of nanocarriers through mucus and reversibly opens the tight junction on epithelial cells making the absorption of nanocarriers via the paracellular pathway. It was hypothesized that during the penetration of epithelium PC6 coating could be uncoated making the chitosan nanoparticles with insulin reach the blood. This makes the absorption of insulin easy to blood [109] (Fig. 11).

4.4 Mesoporous silica nanoparticles as oral drug delivery nanocarriers

Extensive porous structure of mesoporous materials imparts high adsorption ability and stability to the loaded drugs. Drug delivery kinetics can be controlled by controlling the pore dimensions. Wang et al. showed that mesoporous silica nanoparticle (MSNs) can be used as nanocarriers to improve the oral bioavailability and permeation of poorly

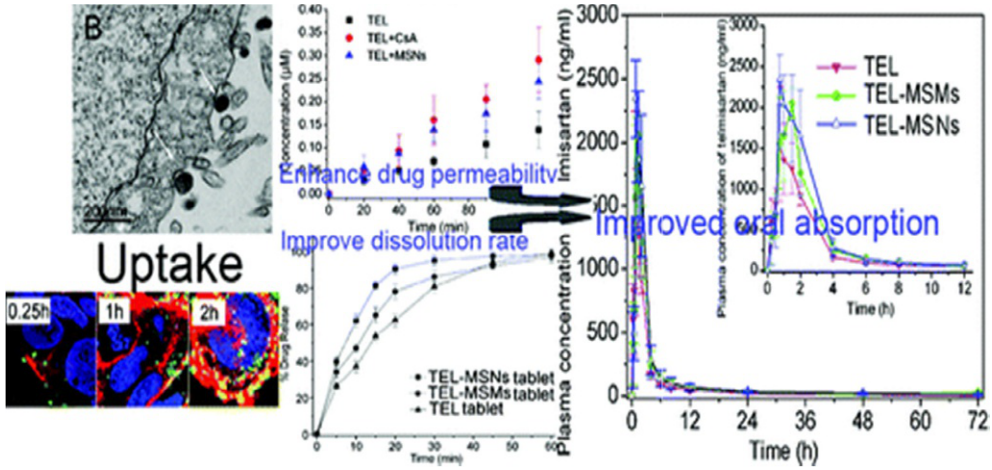

Fig. 12 Improved solubility, permeation, and oral absorption of telmisartan by mesoporous silica nanoparticles. *(Reproduced with permission from reference Y. Zhang, J. Wang, X. Bai, T. Jiang, Q. Zhang, S. Wang, Mesoporous silica nanoparticles for increasing the oral bioavailability and permeation of poorly water soluble drugs, Mol. Pharm. 9 (3) (2012) 505–513.)*

water-soluble drugs. They encapsulated a model drug telmisartan (TEL) into MSNs and tested the solubility and permeability of the drug in comparison to pure TEL and TEL-loaded Mesoporous Silica Microparticles (MSMs). They found that TEL-loaded MSNs exhibited better dissolution rate and better permeability and improved oral absorption. The improved solubility of TEL-loaded MSNs is maybe because the pore channels of the carriers changed the crystalline state of TEL to amorphous form which they facilitate the reduction in the particle size of the drug. TEL-MSN nanocarriers also showed better permeability and exhibited a reduction in the P-glycoprotein mediated efflux mechanism [110] (Fig. 12).

5. Conclusion

Oral drug delivery is the most preferred drug delivery system owing to its simplicity, cost-effectiveness, being pain-free, high patient compliance, and flexibility in dosage forms. The prerequisite for a drug to delivery orally is it should have significant aqueous solubility which leads to enhanced bioavailability. Along with solubility, it should be stable in the acidic and enzymatic environment of the GIT, should show permeability across the intestinal wall. Conventional oral dosage forms show low solubility and permeability which leads to low bioavailability leads to several side effects. There are different alternative strategies to overcome the abovementioned problems. Nano-size drug delivery system offers a promising alternative strategy to improve the oral bioavailability of many drugs. To design a nanocarrier for oral drug delivery, we need to consider the size, surface

charge, stability, encapsulating efficiency, surface composition, biocompatibility, targeted delivery, toxicity, immunogenicity of the nanomaterial. Polymer-based nanocarriers offer more stability toward biological fluids like acidic and enzymatic environment of GIT whereas lipid-based nanocarriers offer increased gastrointestinal absorption and increased plasma concentration. So, hybrid nanocarriers having both lipids-based and polymer-based components may give desired results in oral drug delivery. We need to choose suitable surface groups on the nanocarriers to overcome mucus and epithelial cell barriers of the intestine which enhances the absorption of the drug in the blood.

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CHAPTER 10

Nanomedicine for inflammatory bowel disease

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1. Introduction

Inflammatory bowel disorder is a disorder of the bowel, which is characterized by a complex, relapsing, and remitting inflammatory condition, associated with Crohn's disease and ulcerative colitis. Although the signs and symptoms are distinct in Crohn's disease and ulcerative colitis, however, there are different clinical characteristics that are similar including relapsing and remitting inflammation of bowel [1, 2]. This chronic inflammatory condition affects mostly the intestine of the infected persons worldwide, where these disorders could be clearly distinguished by the endoscopic and histological appearance, clinical problems and distribution [3]. In Crohn's disease, any region of the entire gastrointestinal tract may be affected; however, the colon and terminal ileum are affected mostly. Alternatively, inflammatory conditions in ulcerative colitis are limited to the colonic environment. These conditions are associated with genetic factors, immune dysfunction, and environmental contributors leading to an alteration in microsomal population within the intestine [4–6]. In general, the relapsing conditions of inflammatory bowel disorder could be controlled by the available therapies; whereas there is no cure available for this diseased condition. In order to control the acute inflammatory conditions, frequently prescribed therapies include steroids; however, chronic treatments of steroids are restricted for their unfavorable adverse effects [7]. Alternate therapies to temporarily control this inflammatory bowel disorder include amino-salicylates, antibiotics, and immuno-suppressive agents, whereas a minimum of one surgical interventions are common in approximately 70% of the inflammatory bowel disorder patients within their lifetime [8, 9]. Different treatments on this inflammatory bowel condition control in a variable manner depending on the severity, stage, location, and phenotype of the disease.

Treatment of this diseased condition appeals delivery of the therapeutics directly to the site of action, i.e., colon, to avoid undesirable systemic exposure and associated adverse effects of the medications. Therefore, conventional treatments are usually restricted for their systemic exposure and associated adverse effects and toxicities. And

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thus, therapies are not found genuinely effective in the control of the disease from its root, and thus the disease has become chronic even with the application of therapeutic interventions. On the other hand, delivery of the drugs directly to the colon is also a challenging platform for the formulation scientists where the therapy has to travel a long way from the oral cavity to the large intestine through oral administration of the medicament. Alternatively, delivery can also be projected through rectal administration; however, that route is not convenient to the patient. Thus, specific delivery of the therapies to the colon through oral administration of the formulation is projected to achieve sufficient concentration of the drug at the colon to obtain desired therapeutic efficacy [10, 11].

The physiological environment favors to release certain medication at the particular site of the colon [12]. For example, amino-salicylate prodrugs (olsalazine or sulfasalazine) activated by the metabolic activity of the microbiomes present in the colonic environment [13]. Delivering therapeutics through controlled release mechanism could be projected to deliver the entrapped therapeutics to the site of action, exposing the systemic circulation minimally. Thus, the use of matrix system for prednisolone sodium metasulfobenzoate with polysaccharide coatings has shown to release the entrapped drug within the colon due to enzymatic degradation of the polymer by the bacteria present in that environment [14]. Recent formulation researches are focusing on the use of natural gums, which will release the therapeutic agents preferentially to the site of action [12]. Other approaches also include solubilization of coated polymer of the formulation at the specific pH of the colon, or a time-dependent release of the formulation to allow the release of the drug to obtain therapeutic efficacy [1]. This chapter has focused to summarize the published literature to provide a platform to the readers on colon-specific delivery of therapeutics in the effective control of inflammatory bowel disorder.

2. Physiological considerations in colonic drug delivery

In continuation to the previous discussion, it could be clearly understood that delivery of the drugs specifically to the colon needs to cross various physiological barriers to ensure effective delivery to obtain optimal efficacy of the therapeutic following oral administration. The physiological factors in colon-specific delivery have been depicted in Fig. 1, which could provide a guide to design a formulation for the improvement of residence time [1]. Therefore, the following factors must be considered when a formulation is designed to target colonic delivery:

- **i.** location of the colon at the distal part of the gastrointestinal tract is the major challenge;
- **ii.** residence time of the formulated delivery system within the gastrointestinal environment;
- **iii.** impact of gastrointestinal environment on the delivery system;

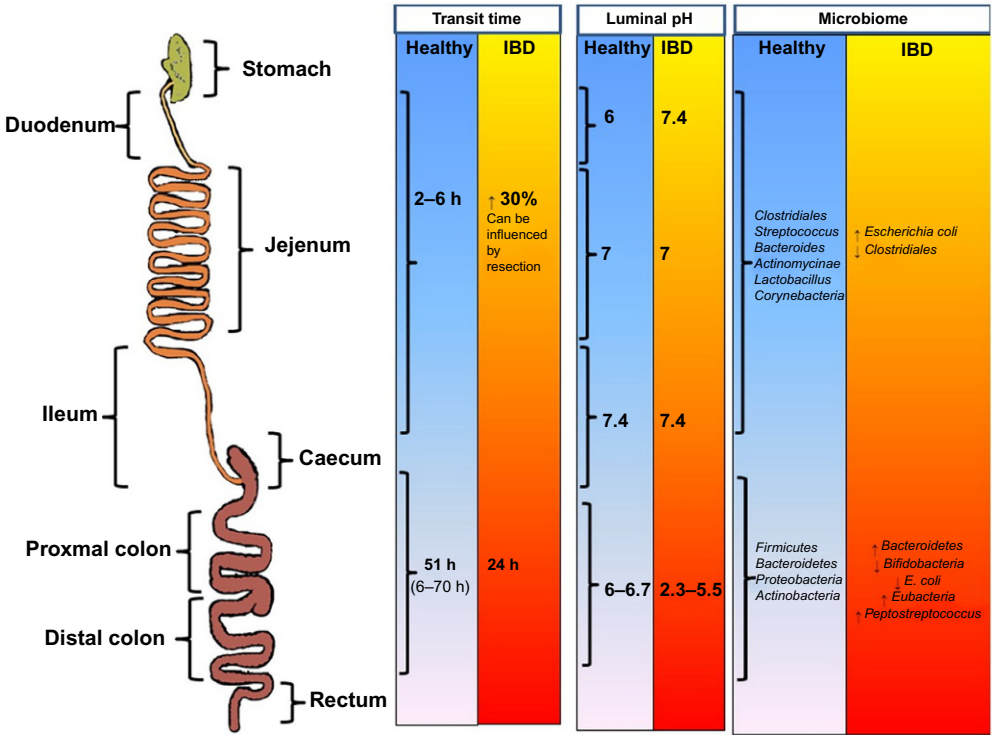

Fig. 1 Physiological and microbial changes to the gastrointestinal tract in inflammatory bowel disease [1].

iv. dissolution pattern of the formulation to release the incorporated drug at the site of action;

v. the volume of the gastrointestinal tract and the presence of food particles; and

vi. metabolism probability of the incorporated drug or the formulation within the gastrointestinal environment due to the effect of microbial degradation or enzymatic effect [1, 15].

Therefore, the transit time of the formulation is an important consideration to ensure effective delivery of the formulation to the site of action [15]. To elaborate, transit time for the gastrointestinal content within the small intestine is about 4 h, where the individual variability may range from 2 to 6 h [16]. Alternatively, the transit time of the content in the colon varies greatly, ranging from 6 to 70 h as reported in the literature [1, 17]. Further, this transit time is also influenced by the gender, where longer transit time in the colon has been evidenced in females [18] and on the other hand, individual's bowel movements also evidenced this transit time and thus affected by the time of dosing [19].

It is important to bring into the discussion that the pH of the gastrointestinal environment is altered during Crohn's disease or ulcerative colitis, as this condition affects the pH minimally to the small intestinal environment, however, the pH of the colon is known to decrease significantly [20]. Such alteration in pH and motility of the gastrointestinal tract affect negatively the small intestinal bacterial overgrowth; however, the large intestine is highly affected [21].

3. Alteration of gastrointestinal physiology in inflammatory bowel disorder

In this section of the chapter, we will elaborate on the consideration of changes in the physiological state of the patient with inflammatory bowel disorder. Based on the previous discussion, it is obvious to consider the changes in transit time and gastrointestinal motility, because alteration of motility and diarrhea consequently affects the pH, mucosal integrity, microbiome environment, and intestinal volume (Fig. 1). Overall, the gastrointestinal physiology is altered in inflammatory condition, which ultimately affects the targeting approach of the contemporary formulation approaches.

3.1 Transit time

The time to reach the chyme following absorption in the small intestine to the caecum is called orocecal transit time. This orocecal transit time varies greatly in inflammatory bowel conditions, where it is known to be delayed in Crohn's disease or ulcerative colitis as compared to transit time in healthy volunteers [22]. Concurrently, change in the microbiome environment of the small intestine (small intestinal bacterial overgrowth) in the inflammatory bowel disease patients leads to the increase in orocecal transit time [23].

Alternatively, transit time in the colon was observed to be faster in inflammatory bowel condition, leading to the common diarrheal complaints by the patients [24]. And thus, recurring diarrhea along with bloody diarrhea are the debilitating and the most predominant symptoms in inflammatory bowel disorder condition. This multifactorial pathogenesis of inflammatory bowel disorder may lead to the mucosal impairment of the intestine, resulting in impairment of barrier function with persistent inflammatory response of the bowel. Thus, the pathogens directly invade the intestine and alter the normal function of the intestinal tract. Therefore, reabsorption of fluid and electrolytes is hampered, leading to intestinal water accumulation and electrolyte retention, leading to diarrheal condition. Further, the invasion of the microbiome within the gastrointestinal wall-promoted dysbiosis leads to bloody diarrhea and impairment of the immune system [24]. Thus, delivery of the drug formulation in the colon may not release the therapeutic agent at the right site at the right time. Studies have revealed longer retention of the delayed-release formulation in the proximal part of the colon, where drug

concentration was found to be minimal in the distal part [25]. Therefore, consideration of transit time is an important parameter when the formulation approaches in the colon for site-specific delivery and treatment.

3.2 Alteration of intestinal pH

The colonic pH of the patients with Crohn's disease or ulcerative colitis is known to be altered, reduced significantly, however, alteration in small intestinal pH is not evidenced in the literature [26]. Alteration of intestinal volume, electrolytes, microbial alteration leading to fermentation processes, metabolism of fatty acids by the bile salts, and lactate secretion is known to play a major role in alteration of the intestinal pH in inflammatory bowel disorder. Movement of acidic chyme through the small intestine (pH 6.8) to proximal colon (pH 7.2) is maintained in normal condition; however, alteration of above factors changes the pH drastically towards acidic side. In a study, the pH range of the patients with ulcerative colitis depicted between 2.3 and 5.3 [27, 28]. Alternatively, irrespective of the disease condition, an average pH range of 5.3 had been reported in patients with Crohn's disease [29]. Alteration of pH of the colonic environment alters the microbiome environment of the intestine. Such alteration may further impede the release of drugs from pH-targeted deliveries through the removal of pH-dependent coating of the formulation or formulation is expected to be released following enzymatic degradation within the colonic environment.

3.3 Volume of intestinal content

Damage of mucosal layer along with the alteration of the electrolytes and water reabsorption from the large intestine leads to increased load. Such a condition consequently alter the intestinal transit time, microbial metabolism, and pH of the environment. An increased volume of the intestinal mass alters the enzymatic concentration to that area, changing intestinal transit time, enzyme-dependent metabolism of the polysaccharides and carbohydrates [1]. Therefore, increased volume of the intestine will definitely alter the conventional formulations delivered to the colon for remedy.

3.4 Mucosal integrity

Transportation of small molecules (e.g., water secretion and luminal glucose, ions, leukocytes, and cytokines transportation) is a normal phenomenon from the epithelial barrier of the large intestine, whereas large molecular transportation is completely abolished due to tight junctions, the apical transmembrane protein complexes [30]. Inflammatory condition of the colon in inflammatory bowel disease is known to alter mucosal integrity, and simultaneously hamper mucosal metabolism because of the physiological attempts to limit further damage [31]. The presence of P-glycoprotein (P-gp), the drug efflux pump, and high viscous mucous gel layer of the intestine affect permeation of P-gp substrates,

and lipophilic drugs and mucoadhesive formulations, respectively. This phenomenon can be well explained by the expression of multidrug resistance gene in the development of steroid resistance while treating irritable bowel disorder with glucocorticoids [32]. Nanotechnology has brought novel formulations which have shown to overcome the biological barrier of multidrug resistance and make the entrapped drug available to the site of action [33] .

4. Nanotechnology in therapy

Nanotechnology has brought several novel deliveries within the nanometer size range to passively and actively target the site of action. These formulations are considered as potential gadgets with a wide array of application in complicated deliveries of pharmaceutical formulations. The advantages of these nanocarriers include solubility enhancement, stability improvement, and simultaneously enhancement of bioavailability, to induce passive drug targeting as well as lower systemic toxicity of the therapeutics [34, 35]. These nanocarriers are found to circumvent the issues with conventional deliveries in pharmaceutical applications because of their small size, which ultimately results in better bioavailability, effective targeting at the diseased tissues with more safer deliveries [36, 37]. Such improved efficacy of the nanocarriers has shown to produce enhanced control of the diseased state over the conventional therapies with decreased dosage [38, 39]. In this section of the chapter, we will elaborate on different nanocarrier systems with improved therapeutic deliveries in the management of inflammatory bowel disorder.

4.1 Polymeric nanoparticle

Polymeric nanoparticles (NPs) are nano-sized colloidal nanocarriers prepared either from natural or synthetic polymers with sizes between 10 and 1000 nm. Based on the method of preparation, polymeric NPs are classified into two types: nanospheres and nanocapsules. Nanospheres are matrix systems in which the drug is uniformly dispersed, whereas, in nanocapsules, the drug is encapsulated within a polymeric membrane. Polymeric NPs offer several advantages over other technologies in delivering drug orally. Mostly polymeric NPs used in oral drug delivery are biodegradable, biocompatible, and nontoxic. Numerous polymers including chitosan, gelatin, alginate polylactic acid (PLA), polyglycolic acid, poly (lactic-*co*-glycolic acid) copolymer (PLGA), poly(methylmethacrylate), poly(alkylcyanoacrylate), and poly(butyl)cyanoacrylate have been used in the preparation of polymeric NPs. PLA and PLGA are biodegradable polymers approved by the US Food and Drug Administration for human use.

Polymeric NPs offer several advantages for oral drug delivery over other technologies. They can prevent enzymatic degradation, can protect the encapsulated drug from unfavorable pH conditions and improve the drug stability in the gastrointestinal environment.

4.1.1 pH-sensitive delivery

Though the oral route is the most preferred route of drug delivery, conventional oral formulations have limited use in treating inflammatory bowel disorder as it suffers from many challenges and obstacles like acidic and enzymatic drug degradation, high first-pass metabolism and permeation which finally affect drug absorption. Hence, there is a need to develop drug delivery systems (DDS) that can deliver drugs specifically to the inflamed regions of the colon in case of inflammatory bowel disorder. The pH-dependent DDS with pH-dependent polymers is an effective approach to target the drugs to the colonic region. The polymers which are widely used for colon targeting are polyacrylic acid, methacrylic acid, polymethacrylic acid, and their derivatives which dissolve at a high colonic pH ($\geq$6.8) [40, 41]. Methacrylic acid copolymers, which are commonly known, as Eudragits are available in different grades, which depends on the ester: carboxylic acid functional group that dictates the solubility at the equivalent pH. Exposed to specific pH conditions (6.8–7.4) lead to deprotonation of the carboxylic acid group, subsequent swelling, followed by polymeric erosion, dissolution, or a combination thereof resulting drug release [42]. In dextran sulfate sodium-induced mouse colitis model, Eudragit S100 coated PLGA NPs of budesonide have shown a significant reduction in inflammation [43]. Similarly, pH-sensitivity release was observed for Eudragit S100/PLGA NPs loaded with curcumin and further, noticeable reduction of the TNF-α secretion in lipopolysaccharide activated macrophages was reported [44].

A hybrid approach using enzyme and pH-sensitive nanocarrier system using enzyme-sensitive azopolyurethane polymers the pH-sensitive Eudragit S100 has been studied. Coumarin-6-loaded nanospheres were developed using pH-sensitive Eudragit S100 alone and in combination with enzyme-sensitive azopolyurethane. A burst release of the drug at neutral pH in rat colitis model was observed with NPs developed using pH-sensitive polymer alone, whereas, more controlled drug release with sustained-release pattern with 5.5 times higher accumulation in the inflamed colon tissues was reported with hybrid nanosphere using pH-sensitive and enzyme-sensitive polymer [45]. Similarly, in an in vivo study, greater accumulation of budesonide was reported from hybrid nanosphere of budesonide in the inflamed tissues as compare to nanosphere with simple Eudragit S100. Therefore, it can be inferred that enzyme-sensitive polymer plays an important role in achieving a controlled release of the drug and improving selective targeting to the inflamed colon tissues [46].

4.1.2 Particle targeting

Over the past few decades, inflammatory bowel disorder is treated by administration of antiinflammatory medications like aminosalicylates, corticosteroids, antiTNF-α monoclonal antibodies, and immunosuppressants [47, 48]. However, these medications need high daily doses and present serious adverse events either due to their unspecific efficacy upon administration or systemic absorption [49–51]. The medication-related side effects

can be reduced by selectively targeting the drugs to the inflamed colonic region to increase the therapeutic efficacy towards inflammatory bowel disorder [52, 53] Nano-sized particles have been found to specially accumulate in inflamed regions. Localized delivery of NPs loaded with antiinflammatory drugs to the inflamed colonic region is of the important step to achieve in the targeting of inflammatory bowel disease. Moreover, the drug release can be triggered by either exogenous stimuli like heat, light, and ultrasound or endogenous stimuli like pH, oxygen, and enzyme concentrations [54].

Polymeric NPs impart considerable flexibility in design by enabling modification of physicochemical properties, e.g., surface charge, size, and hydrophobicity, as well as drug release characteristics like a controlled release or triggered by external stimuli. In addition to these, the surface properties can be modified by using conjugating different polymers to the NP surface [55, 56].

Physicochemical parameters of NP can be modified to target inflamed intestinal tissue. Lamprecht and team reported that smaller NPs sizes were more effective in regard to bioadhesion to the inflamed tissue in a rat inflammatory bowel disorder model [57]. Further, a better deposition was investigated in thicker mucus and ulcerations regions within the inflamed tissue. Similar results have been reported for many drugs including 5-aminosalicylic acid [58], FK506 (tacrolimus) [59], and dexamathesone [60], in rodent inflammatory bowel disorder models.

Peptides, antibodies, or small molecules can be attached to the surface as targeting ligands as well to achieve specific interactions with cellular receptors or tissue components [61]. Laroui et al. observed a reduced inflammation as well as loss in body weight when the surface of PLA-polyethylene glycol (PEG) NPs containing TNF-α siRNA is conjugated with Fab portion of the F4/80 antibody compared to control groups [62]. In another study, Moulari and team reported that when PLGA NPs are conjugated with peanut and wheat germ lectins, an increased adherence of NPs to inflamed regions of the intestine is observed resulting in improved clinical activity scores in murine colitis models [63].

In another study, Wilson et al. formulated thioketal NPs using a novel polymer poly-(1,4 phenyleneacetone dimethylene thioketal) which can degrade in the presence of reactive oxygen species (ROS). As high levels of ROS are found at the inflammatory site, NPs composed of thioketal polymers would degrade at inflammation sites following oral administration and subsequently release the drug. When thioketal NPs were encapsulated with TNF-α siRNA and administered to mice with induced colitis, it was observed that the inflammation and body weight loss was reduced over 7 days [64]. Few of the nanoparticular delivery system to target inflammatory bowel disease have been tabulated in Table 1.

Table 1 Nanoparticulate drug delivery in inflammatory bowel disease.

Sl No	Formulation	Polymer	API	Size (nm)	Surface charge	Objective	Results	Source
1	Nanostructured chitosan-based composites	Chitosan	Resveratrol (RES)	108–121	–	• To encapsulate RES into biocompatible non- and cross-linked poly (2-hydroxyethyl methacrylate)-based NPs • To optimize the conditions for RES-loaded Chitosan-NP hydrogels in sustaining drug release.	• The enhanced RES encapsulation efficiency values were achieved in at a RES/polymer ratio of 0.75:1 w/w. • RES-loaded chitosan-NP hydrogels, formulated at 37 °C, 4% chitosan and 10% of cross-linker has shown a marked decrease in drug release rate from the formulation.	[65]
2	nanoparticles (NPs)	Polyesterurethane (PU) and PEGylated form of PU	Infliximab (INF)	1537.33 ± 120.71 (PU-INF) 1706.50 ± 659.73 (PU-PEG-INF)	−2.52 ± 0.30 (PU-INF) −2.03 ± 0.25 (PU-PEG-INF)	• This study was planned to evaluate the PU and PU-PEG potential to act as INF nanocarriers for the treatment of inflammation in an in vitro intestinal epithelial barrier model.	• Developed PU and PU-PEG NPs had shown highest cellular interaction, uptake, and permeability across inflamed epithelial Caco-2 cell monolayer, resulting reduction of IL-8 and inflammation.	[66]

Continued

Table 1 Nanoparticulate drug delivery in inflammatory bowel disease.—cont'd

Sl No	Formulation	Polymer	API	Size (nm)	Surface charge	Objective	Results	Source
3	Folate-functionalized PLGA/PLA NPs	PLGA/PLA	6-Shogaol	246.4 ± 0.8 (PEG-NPs) 249.6 ± 1.3 (PEG-FA-NPs)	−29.37 ± 1.58 mV (PEG-NPs) −24.17 ± 0.41 mV (PEG-FA-NPs)	• To develop novel delivery systems to deliver drug specifically to the inflamed tissue associated with colitis.	• Efficient receptor mediated cellular uptake by colon-26 cells and activated Raw 264.7 macrophage cells • Excellent in vitro and in vivo biocompatibility • Hydrogel system of NPs-PEG-FA/6-shogaol significantly lessen colitis symptoms and hasten colitis wound repair in DSS-induced mouse colitis model	[67]
4	pH sensitive lipid NPs	Polyethyleneimine (PEI) Eudragit S100	Budesonide	302 ± 13	−30 ± 5.3	• To develop sustained and targeted pH sensitive Eudragit S100-coated polyethyleneimine-based lipid NPs delivery to the inflamed regions of the colon.	• Coating the NPs with Eudragit S100 prevented the unwanted burst drug release in stomach and small intestine. • pH-triggered charge reversal of Eudragit-coated lipid NPs facilitated their accumulation in an inflamed colon which is revealed in a DSS-induced colitis mouse model.	[68]

5	Spherical polymeric nanoconstructs (SPNs)	Carboxylterminated poly (lactic-*co*-glycolic acid) (PLGA) and 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC)	Dexamethasone	162.0 ± 8.2	−39.5 ± 2.31 mV	• To evaluate the capability of delivering DEX-SPNs to the inflamed regions and further preventing localized and systemic inflammation both.	• DEX-SPNs are shown to deposit within the hyper-permeable inflamed intestine. • As compared to treatments of free DEX, DEX-SPNs reduce the macrophage infiltration, inflammatory cytokines expression, histological scoring, and rectal bleeding and improve weight loss.	[69]
6	Lipid-based nanocarriers Self-nano emulsifying drug delivery systems (SNEDDS), Nanostructured lipid carriers (NLC), and Nanocapsules (NC)	SNEDDS: LabrafilM2125CS, Labrasol; CremophorEL NLC: PrecirolATO5, Miglyol 812 N/F, Tween 80, KolliphorP188 NC: Protamine	Curcumin	SNEDDS: 49.2 ± 1.2 NLC: 280 ± 8 NC: 188 ± 3	SNEDDS: – NLC: −10.6 ± 0.3 NC: +18 ± 8.7	• To study the combination of lipids and antiinflammatory drug within a nano-delivery for overcoming IBD.	• Higher permeability (around 30-fold) of curcumin across Caco-2 cell monolayers was explored with NC compared to SNEDDS. • Curcumin NLC and curcumin SNEDDS significantly decreased secretion of TNF-α by LPS-activated macrophages (J774 cells).	[70]

Continued

Table 1 Nanoparticulate drug delivery in inflammatory bowel disease.—cont'd

Sl No	Formulation	Polymer	API	Size (nm)	Surface charge	Objective	Results	Source
							• In vivo studies revealed that only curcumin NLC significantly reduced TNF-α secretion and neutrophil infiltration and consequently, colonic inflammation	
7	Self-assembled NPs	Amphiphilic hyaluronic acid	Budesonide	177 ± 6 to 266 ± 16	-3.98 ± 0.46 to -19.66 ± 0.74	• To develop self-assembled hyaluronic acid-coated NPs loaded with budesonide for targeting to the inflammation in intestinal mucosa	• An enhanced CD44 receptor-mediated cellular uptake in inflamed CACO-2 cells was observed. • Reduction in secretion of inflammatory cytokines, e.g., IL-8 and TNF-α from inflamed CACO-2 cells was observed which established antiinflammatory efficacy of budesonide-loaded NPs.	[71]

8	pH-sensitive polymeric NPs	Poly(lactide-*co*-glycolide) acid (PLGA); Polymethacrylate polymer (Eudragit1 S100)	Curcumin	116 ± 3	−40.4 ± 0.6 mV	• To evaluate curcumin release via PLGA/Eudragit1 S100 pH-sensitive polymeric NPs in a selective colonic environment in IBD model	• Curcumin NPs significantly improved permeation of curcumin across Caco-2 cell. • Curcumin NPs significantly reduced secretion of TNF-α via LPS-activated macrophages (J774 cells). • In vivo studies revealed that curcumin NPs significantly decreased neutrophil infiltration and TNF-α secretion while maintaining the colonic structure in a murine DSS-induced colitis model.	[44]
9	pH sensitive-coated NPs	Poly(lactic-*co*-glycolic) acid	Budesonide	200 ± 10.1 (plain NP) 240 ± 14.7 (coated NP)	0 mV (plain NP) −30 mV (coated NP)	• To investigate the beneficial effect of budesonide-loaded nano-delivery for the treatment of IBD.	• Protection of early drug release at acidic pH and assisting drug releases at the neutral to slightly alkaline pH was achieved via pH-sensitive coating.	[43]

Continued

Table 1 Nanoparticulate drug delivery in inflammatory bowel disease.—cont'd

Sl No	Formulation	Polymer	API	Size (nm)	Surface charge	Objective	Results	Source
10	Nanospheres	Chitosan (CS)-modified poly(D,L-lactide-*co*-glycolide) (PLGA) nanospheres (NS)	nuclear factor kappa B (NF-kB) decoy oligonucleotide	366.6	13.2 mV	• To study the antiinflammatory effects of CS-PLGA NS loaded with NF-kB decoy ODN in DSS-induced colitis rat model.	• The coated NPs were shown to be effective in tri-nitrobenzene sulfonic acid (TNBS), dextran sulfate sodium (DSS) and oxazolone colitis mice models in treating IBD. • Effectiveness of oral NF-kB decoy ODN-loaded CS-PLGA NS in treating DSS-induced colitis was established.	[72]

4.2 Solid lipid NP

A new generation of the colloidal carrier of surfactant-stabilized lipids has been introduced in 1991 where the lipids remain in solid state at the storage and body temperature [73, 74]. Such a formulation combines the advantages of emulsions, polymeric NPs, and liposomes where they assemble to contain a hydrophobic lipidic core with phospholipid coating within the size range of 50–1000 nm, at the same time curtailing few of their individual disadvantages through improving the delivery potential of various pharmaceuticals [74]. This assembly of the hydrophobic core is stabilized by the coat of phospholipid, where the hydrophobic tails are implanted into the core lipid matrix, and the hydrophobic region is exposed to the media, making the lipidic formulation hydrophilic (Fig. 2).

As we have discussed earlier that the antiinflammatory agents and the immunosuppressive agents are the drug of choice for the treatment of inflammatory bowel disease, Serpe et al. [75] developed a solid lipid nanoparticular delivery of dexamethasone and butyrate, antiinflammatory agents [69, 76] to improve the oral delivery of the incorporated drugs. Experimental results of the solid lipid nanoparticular delivery of butyrate (cholesteryl butyrate) revealed a significant decrease in different inflammatory mediators (IL-1β, TNF-α) and increase in antiinflammatory cytokines (IL-10) in patients with inflammatory bowel disease when compared with the highest dose of butyrate alone. Likewise, delivery of dexamethasone through this solid lipid NP had revealed similar results in the reduction of the inflammatory condition in inflammatory bowel disease. This study reflected the potential of solid lipid NP delivery system in inflammatory bowel disease [75]. A similar study by Dianzani et al. had focused to improve the delivery of antiinflammatory agents through the development of dexamethasone cholesteryl butyrate-solid lipid NPs [77]. The in vitro and in vivo results of the developed formulation was comparable to the results obtained by Serpe and team [75]. The lowest

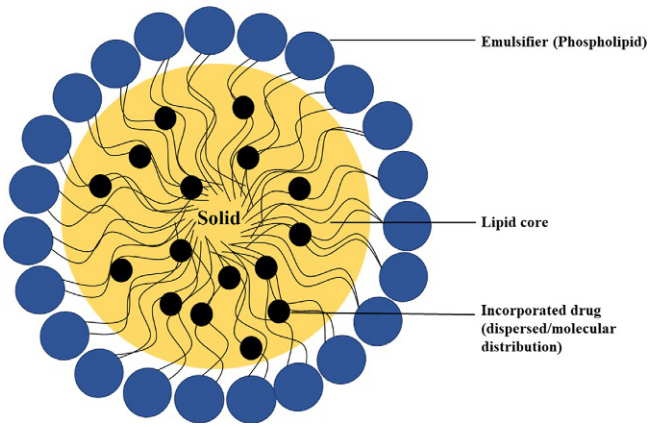

Fig. 2 Schematic representation of the solid lipid NP structure.

concentration of the two antiinflammatory agents through this solid nanocarrier was found to exert more than additive effect when compared with combined individual effects of the drugs. Thus, coadministration of the two antiinflammatory agents in the same lipid-based formulation would always be beneficial in treating inflammatory bowel disease [77].

Yadav and team had approached the delivery of a natural compound, curcumin, through this solid lipid nanoparticular platform. In order to obtain a colon-specific delivery of the formulation, this poorly water-soluble natural compound was incorporated in the solid lipid microparticles to target antiinflammatory response of curcumin. On the evaluation of antiinflammatory and antiangiogenic activity of the formulated lipid carrier in rat colitis and chick embryo models, the formulation was found to possess potent angio-inhibitory activity. Additionally, treatment of the solid lipid NPs of curcumin revealed a quicker weight gain in the experimental animals when compared to control groups treated with dextran sulfate solution. Thus, it has been proved that there was a significant attenuation of the colitis condition induced by dextran sulfate solution [78].

In a similar approach to improve the delivery of budesonide, aiming to get accumulated at the site of inflammation of inflammatory bowel disease patients, the budesonide-loaded nanostructured lipid carriers (NLC) were formulated and characterized with a particle size of 284.0 ± 4.53 nm, where the drug entrapment efficiency was reported as $92.66 \pm 3.42\%$. Developed lipid nanocarrier was further coated with Eudragit S100 to form the delayed-release pellets, which needed 5 h to gain 15% of weight to release the drug. This 5 h duration is sufficient to deliver the formulation in the colonic area and to release the entrapped drug for superior efficacy and safety [79].

Antiinflammatory effect of curcumin had shown to reduce the clinical relapse cases in dormant inflammatory bowel disease patients, where curcumin possesses its antiinflammatory role through modulation of inflammatory mediators, such as cyclooxygenase, lipoxygenase, NF-κB, TNF-α [80]. However, low bioavailability of curcumin due to rapid degradation and poor aqueous solubility limits its use in such inflammatory conditions. To accomplish the antiinflammatory effect of curcumin, Wang and team had reported the development and evaluation of curcumin solid lipid NPs [81]. In their experiment, the authors reported the intraperitoneal administration of free curcumin and curcumin-loaded solid lipid NPs (30 mg/kg) within the experimental IL-1β firefly luciferase transgenic mouse model prior to administration of lipopolysaccharide (to induce sepsis model). They formulated the curcumin-loaded solid lipid NPs by adopting emulsification and low-temperature solidification methods (Fig. 3). Intraperitoneal administration of the formulation was found to increase the drug accumulation at the site of inflammation due to the enhanced permeation and retention effect of the nanocarrier. In their report, sustained release of the entrapped curcumin through internalization of the formulation by the macrophages and depolymerization effectively reduced expression of IL-1β in groups of animals injected with curcumin-loaded solid

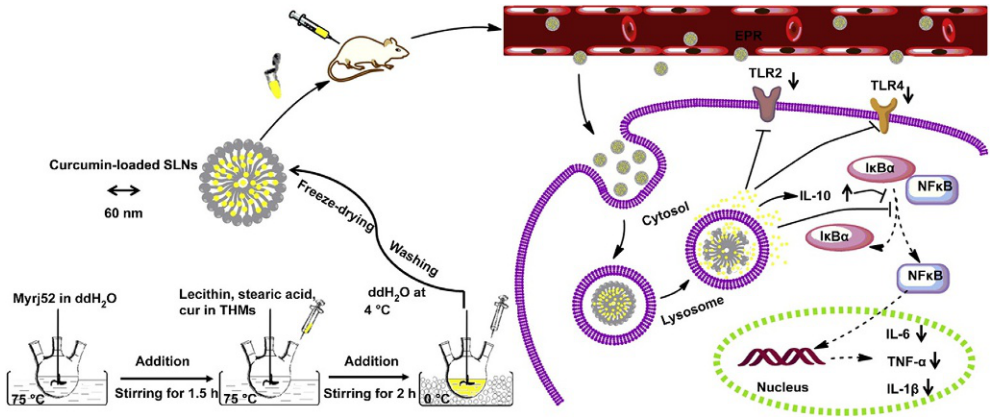

Fig. 3 Schematic diagram shows the mechanisms underlying the ability of Cur-SLNs to overcome the LPS-induced sepsis [81].

lipid NPs when compared to free curcumin-treated group. Further, they also reported the reduction of pro-inflammatory cytokines in the serum of the animals, such as IL-1β, TNF-α, and IL-6, when compared with free curcumin-treated animals. Further analysis revealed that the antiinflammatory response of the formulation is due to the blockade of TLR4/2-NF-κB signaling pathway (Fig. 3) [81].

To extensively evaluate the efficacy of lipid nanocarriers containing curcumin, Beloqui and team formulated three curcumin-loaded formulations, lipid core-shell protamine nanocapsules, NLC, and self-nanoemulsifying drug delivery systems (SNEDDS) [70]. In vitro findings on Caco-2 cell permeability study demonstrated more than 30-fold increase in permeability of lipid core-shell protamine nanocapsules than the SNEDDS, where NLC and free carriers of curcumin showed decreased permeability compared to the other two. However, when the same formulations were tested in vivo model, the NLC showed a significant decrease in colonic inflammation through the reduction in TNF-α secretion and neutrophil infiltration in an experimental model. This reversed result from the permeability study has been explained by the increased retention of curcumin to the affected area due to enhanced retention and permeability effect and, thus, prolonged action to control the inflammation more effectively [70].

With the aim to improve therapeutic targeting of dexamethasone, ultra-small solid archaeolipid NPs were formulated by another group. The researchers demonstrated that the activity of the entrapped drug strongly reduced inflammatory cytokines, such as IL-12, IL-6, and TNF-α in lipopolysaccharides induced J774A1 cell lines. This could be inferred that the lipidic formulation would be suitable for oral-targeted colonic delivery to inflamed mucosa [82].

4.3 Other nanocarrier formulations for potential delivery in inflammatory bowel disorder

4.3.1 Nanoemulsion

Nanoemulsions are thermodynamically stable dispersion of oils in water, where a thin layer of surfactants stabilizes the dispersed droplets. These formulations are gaining tremendous interest to deliver the hydrophobic therapeutics within the lipidic core of the formulation, whereas the physicochemical properties of the formulation make them more compliant to the patients [83–85]. Deliveries of such formulations are also found to be safe, as depicted in previous studies in the literature [86, 87]. In order to overcome the solubility, and also the low bioavailability effect of curcumin, nanoemulsion strategy is adopted by Young and team [88]. The authors evaluated the efficacy of curcumin nanoemulsion in a novel transgenic animal model harboring an NFκB-luciferase reporter gene. Bioluminescent imaging of the experimental animals following the administration of nanoemulsion formulations and free drug and lipopolysaccharide demonstrated effective suppression of NFκB activity. Oral administration of curcumin nanoemulsion depicted a decrease in macrophage migration via NFκB and MCP-1 inhibition [88]. The role of this nanoemulsion in inflammatory bowel disorder has been extended to diagnose the disease effectively. With the aim to deliver perfluorocarbon, it was emulsified and reduced the size to less than 200 nm to improve circulatory time through enhance permeation and retention effect, where this distinct size would prevent their recognition by the reticuloendothelial cells. Further modification of the carrier surface with PEG has shown to improve circulation via abolishing cellular uptake of the formulation [89].

4.3.2 Micelles

Distinctive amphiphilic molecules in aqueous environment formulate another nano-sized colloidal dispersion, i.e., micelles. The hydrophobic tails and hydrophilic heads are rearranged in the aqueous environment to form a hydrophobic core, which can be used as a reservoir for the hydrophobic therapeutics [90]. On the other hand, the hydrophilic surface of the micelles helps to stabilize the formulation in the aqueous environment. Usually, these nano-sized micellar dispersions are formed upon increasing the concentration of the amphiphilic molecules (e.g., polymer) above critical micelle concentration, with a great possibility to modify the surface of the formulation [90]. To establish the activity of the micellar delivery, styrene maleic acid micelle was prepared to encapsulate raloxifene. Treatment with raloxifene-loaded styrene maleic acid micelle in U-937 cell lines has shown to enhance downregulation of the macrophage inflammatory proteins (MIP1α) and inflammatory cytokines (IL-6, IL-1β, and TNF-α) compared to the free drug. In vivo activity of the formulation and raloxifene has shown to increase the body weight of the experimental Balb/c mice following a treatment of 14 days when compared to animals in control group. Further, the length of the colon increased in control groups of animals with the smallest size was observed in raloxifene-loaded micelle.

Further, treatment of developed formulation also reduced the diarrheal condition in the experimental animals [90].

In another study with 4-Amino-6-hydroxypyrazolo[3,4-*d*]pyrimidine (AHPP), a xanthine oxidase inhibitor to target generation of superoxide anion (O_2^-) generated by xanthine oxidase, is incorporated in the micellar delivery system. The activity of xanthine oxidase inhibitor is found to benefit inflammatory bowel disorder [91]. The experimental results suggested that intravenous or oral administration of PEG-AHPP showed a marked reduction in diarrheal condition as well as the length of the large intestine. Increased level of inflammatory cytokines (IL-6 and TNF-α) because of dextran sulfate sodium treatment was found to be significantly decreased [91].

4.3.3 Liposome

Liposomal deliveries are known as vesicles of a phospholipid, where the bilayer of the phospholipid encloses a separate aqueous phase. Unique properties of these liposomal formulations to entrap hydrophilic therapeutics within the core and hydrophobic components on the lipidic bilayer made them popular dosage form [92]. These liposomal deliveries are well investigated and common particulate delivery to provide improved therapeutic efficacy over a wide variety of pathophysiological conditions through overcoming obstacles by the biological barriers, improving bio-distribution of the therapeutics, uptake by the tissues, and finally, the stability of the compounds [92, 93]. Options to modify the surface of the liposomal delivery increase their passive targeting as well as active targeting potential to obtain an improved therapy [94].

Accumulation of liposomal deliveries is an important aspect, and the same has been revealed through the formation and delivery of fluorescence-labeled liposome. In a research, it has been revealed that the present macrophages in the inflamed area of colon attract this lipidic delivery, and through accumulation results in increased drug concentration to that particular area for an improved treatment option for this disease [95]. An approach was made to incorporate 6-mercaptopurine within the lipidic membrane of the vesicles and 5-aminosalicylate to the core of the liposomal delivery to target inflammatory bowel disease [96]. Such entrapped delivery of 5-aminosalicylate has shown reduced systemic exposure of the drug. Further, liposomal adherence at the colonic cite resulted in increased local drug concentration of 5-aminosalicylate, however, the local tissue concentration of 6-mercaptopurine has not improved compared to drug solution [96].

Experimental findings provide evidence of nano-particulate formulations in the improvement of deliveries for better therapy in inflammatory bowel disease patients.

5. Conclusion

An improved therapeutic efficacy of drugs in several diseased conditions can be achieved by the nanoparticular delivery systems, where the nano-ranged size of the formulation allows prolonged circulation within the system through enhanced permeation and

retention effect, whereas possibility to modify the surface can improve target ability of the formulation through active targeting. Accumulation of these formulations to the site of large intestine showed site-specific delivery of the drugs, without exposing the systemic circulation much. This provides the facility of improved efficacy and safety to the patients.

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CHAPTER 11

Nanomedicine for vaginal drug delivery

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1. Introduction

Nanocarriers, defined as drug vehicles in the submicron size, have been under extremely extensive research in an attempt to fundamentally change the conventional strategies for drug administration. Realistically speaking, drug delivery nanosystems have brought about a plethora of distinctive qualities making them capable candidates of reshaping the future of medicinal therapy. Recent advances in pharmaceutical nanotechnology have allowed the creation of versatile nanosystems, such as nanoparticles, vesicles, and nanoemulsions that can be tailor engineered to overcome barriers imposed by the organism and deliver their therapeutic cargo in temporally and spatially controlled manner enabling deep penetration into body tissues, efficient internalization at the cellular level, and controlled drug release.

Any pharmacologically active substance regardless of its molecular weight, hydrophilicity, stability, or any other physicochemical properties, including, small molecule drugs, peptides, proteins, and deoxyribonucleic acid (DNA)/ribonucleic acid (RNA), can be fairly easily loaded into a suitable nanosystem for introduction by almost any route of administration in order to achieve efficient delivery to the site of interest. Hence, superior chemical and biological stability, ability to shield the loaded drug from degradation sources, smartly tuned drug pharmacokinetics, and biodistribution are a few examples to name among the unique features offered by these delivery platforms.

While one aspect of engineering an ideal nanosystem deals with enhancing drug loading capacity, it more importantly involves the optimization of system characteristics that determine its biological performance and fate. Thanks to emerging nanotechnology techniques and to the versatility of nano-sized systems, handling particle size and surface characteristics can be a key to control particle-surface interactions, biological circulation time, membrane permeability, drug localization, and drug targeting (passively or actively).

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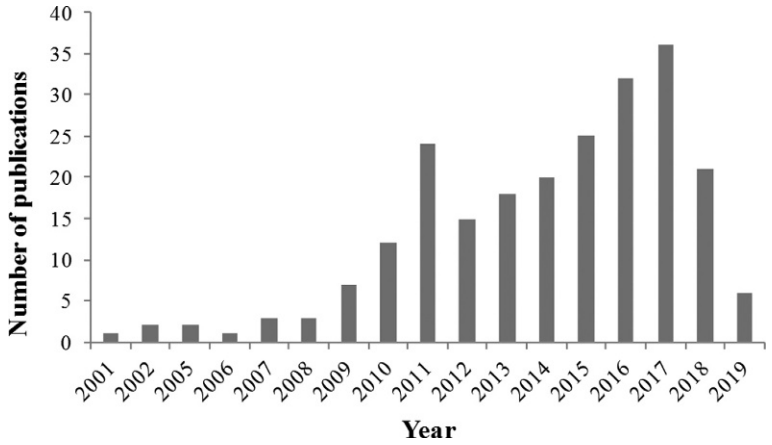

Fig. 1 Publications in the field of nanoparticle-based vaginal systems over the last two decades on PubMed database (as on March 23, 2019). Keywords used for search were: vaginal and nanoparticle.

The steadily growing interest in the use of drug nanocarriers for vaginal administration over the last two decade has been notable (Fig. 1). The cervicovaginal mucosa comprises a complex multilayered environment, which can impede drug transport using conventional drug delivery systems. Nanotechnology offers an effective strategy for vaginal drug administration owing to their special characteristics including sustained drug release, bioadhesion to mucus, and mucosal penetration ability [1, 2]. This chapter is intended to present an update on the recent advances in drug nanosystems for vaginal delivery. An overview of the anatomy and physiology of the vagina, features of vaginal route, and barriers to vaginal drug delivery is provided. In addition, a broad account of various drug nanocarrier for intravaginal administration, including nanoparticles (NPs), liposomes, nanoemulsions, dendrimers, and cyclodextrins, is given.

2. Vaginal drug delivery

Vaginal drug delivery indicates the administration of medications within the vaginal cavity to produce local or, less frequently, systemic pharmacological effects. The vaginal route has been traditionally exploited for the management of local genital conditions such as infections, vaginitis, and labor inducing/prevention purposes. Thus, a variety of pharmacologically active substances, including antibacterials, antifungals, antiprotozoals, antivirals, labor inducers, spermicidal agents, and sexual hormones, have been formulated in vaginal dosage forms [3, 4].

Furthermore, systemic absorption after intravaginal administration of chemicals has been well known for a long time; In particular, low molecular weight (MW), nonpolar drugs are usually well absorbed and able to achieve therapeutic systemic levels. However,

systemic delivery via the vaginal route has not gained interest until recently due to cultural sensitivity and gender specificity [5].

The vagina is advantageous as a route of administration for noninvasive, controlled delivery of drugs intended for both local and systemic effects [1–4, 6, 7]. This route offers a number of benefits over other routs of drug delivery including: (i) avoidance of hepatic first-pass effect because absorbed drugs penetrate directly to the systemic circulation via the inferior vena cava; (ii) prevention of hepatic toxicity induced by some drugs, for example, steroids; (iii) easiness of administration and possible self-insertion and removal of the dosage form; (iv) protection of drug against gastrointestinal enzymatic degradation and interferences with drug absorption and preclusion of side effects associated with oral route; (v) avoidance of parental routes associated inconvenience due to pain, tissue damage, and possible infection; and (vi) "first-pass uterine effect" (preferential distribution to the uterus) due to the rich vascularization between the vagina and uterus which allows uterine targeting of therapeutic agents, such as progesterone and danazol.

Pharmacological performance in the vaginal cavity is largely dependent on the vaginal physiological and anatomical factors. Consequently, an in-depth understanding of these factors is of crucial importance in designing optimal intravaginal drug delivery system.

3. Nano-sized systems for vaginal drug delivery

Nano-sized systems have received considerable attention for vaginal drug delivery due to a wide array of benefits they can offer to address the limitations associated with conventional vaginal dosage forms. Vaginal drug nanosystems can avoid leakage and messiness, produce sustained drug release (and effect), and minimize adverse effects in comparison with conventional dosage forms, thus, enhancing patient compliance. In addition, the use of nanosystems cannot just reduce the influence of vaginal physiological factors on drug efficacy but it can also be exploited for controlled drug delivery, such as to employ pH for selective stimuli-responsive drug release. Furthermore, the encapsulation of the active drug in the nanosystems masks the influence of its physicochemical properties on absorption and pharmacological efficacy. Hence, nanosystems can enhance the dissolution of a hydrophobic drug, and improve their stability by preventing drug hydrolysis and enzymatic degradation. Excitingly, surface functionalization with targeting ligands supplies nanosystems with a great opportunity for selective drug therapy.

In this section, an overview of the state-of-the-art developments in field is presented and the different categories of nanoplatforms currently investigated for vaginal drug delivery including nanoparticles, liposomes, nanoemulsions, dendrimers, and cyclodextrins are discussed.

3.1 Nanoparticles

NPs are the most extensively studied nanocarriers for vaginal drug delivery. Vaginal NPs can be formulated with a variety of materials including biodegradable and biocompatible

polymers, such as poly(lactic-*co*-glycolic) acid (PLGA) and poly(D,L-lactic) acid (PLA), and lipids. Nanotechnology offers potential for developing NPs that provide sustained drug release, exhibit mucoadhesive properties, and/or diffusive ability to quickly diffuse across the vaginal mucus to reach the underlying tissues, thus avoiding clearance mechanisms of the mucus. Recent examples of NP-based systems for vaginal drug delivery are presented in Table 1.

3.1.1 Nanoparticle-related factors influencing vaginal drug delivery

Particle size, formulation components, surface charge, and surface modification functionalizing have been identified as the major factors that determine the success of intravaginal drug delivery using NP-based systems.

Particle size plays a key role in determining the cervicovaginal mucus (CVM) penetration ability and velocity of the nanoparticle. This effect can be illustrated by the behavior of natural infectors like viruses; while small viruses (less than 56 nm in size) can diffuse in CVM as rapidly as in saline, the larger viruses show significantly hampered diffusion—the diffusion of herpes simplex virus (HSV), which has a diameter of 180 nm in size, in CVM was found to be 100- to 1000-fold slower than in PBS [17]. Similarly, it was demonstrated that polystyrene NPs (average diameter ranging between 59 and 1000 nm) adhere firmly to CVM, resulting in complete immobilization [17]. Based on these observations, a mucus mesh spacing of between 20 and 200 nm was suggested concluding that NPs with diameter larger than 59 nm are not likely to penetrate efficiently across the CMV to the underlying tissues [17]. These findings were contested a few years later when a report by Lai et al. demonstrated that polystyrene NPs with hydrophilic surface (modified with PEG, 2 kDa) and diameters of 200–500 nm were able to diffuse through CVM and produced higher diffusion across the CMV relative to unmodified NPs of the same size [18]. Furthermore, smaller NPs (~100 nm in diameter) were found to be slower than larger ones (size: 200–500 nm), which may be explained by the possible entrapment of these particles in small pockets throughout the mucous network. However, it was confirmed that unmodified carboxylated NPs had strong mucoadhesive properties and were not diffusible through the CVM [18].

Different research groups have optimized nanocarrier formulations using Box-Behnken factorial design. In a study by Meng et al., the formulation variables of NPs loaded with tenofovir, an anti-HIV microbicide, fabricated using a gelation method by the addition of sodium triphosphate pentabasic (STP), were investigated [19]. The influence of three formulation factors was considered: chitosan concentration, STP/chitosan weight ratio, and drug/chitosan weight ratio. The optimal formulation based on a mathematical optimization process produced NPs with a size of 208 nm with a relatively low EE% of only ~6%. To improve the encapsulation efficiency, a 50% (v/v) ethanol/water mixture was used as a solvent for chitosan. While the use of ethanol resulted in EE% of 20%, particle size increased to 602 nm. Increased mucoadhesive ability from 6% to 12%

Table 1 Recent examples of nanoparticles for vaginal drug delivery.

Composition	Active agent/ treatment	Preparation method	Mean size (nm)	Final dosage form	Surface modification	References
PLGA	PDGFR-β *si*RNA (condensed by PEI)/ *Chlamydia trachomatis* infection	Double-emulsion evaporation	≈260	–	PEGylation	[8]
Glyceryl monostearate-phosphatidylcholine SLNs	CCR5 *si*RNA/ HIV infection	Emulsion solvent diffusion	≈270	Electrospun porous pH-responsive polyurethane membrane	–	[9]
Silver metallic	Tannic acid/ genital herpes infection	Chemical reduction	≈30	–	–	[10]
Chitosan and dextran sulfate	PCS peptides/ IV vaccination	Polyelectrolyte complexation	≈30	–	–	[11]
PCL	Itraconazole/ vulvovaginal candidiasis	Nanoprecipitation	120 (for NS), 190 (for NC)	Gel (60% Poloxamer 188 and 20% PEG 400)	–	[12]
Precirol ATO 5, oleic acid, and Kolliphor RH 40 nanolipid	Fluconazole/ Vulvovaginal candidiasis	Phase inversion temperature	≈160	Gel (Carbopol 974P; 1%, w/w)	–	[13]
PLA-HPG	Elvitegravir/ HIV infection	Nanoemulsion	≈130	–	Hyperbranched polyglycerols oxidized to aldehydes form bioadhesive NPs	[14]

Continued

Table 1 Recent examples of nanoparticles for vaginal drug delivery—cont'd

Composition	Active agent/ treatment	Preparation method	Mean size (nm)	Final dosage form	Surface modification	References
Cellulose acetate phthalate	Dolutegravir/ HIV infection	Interfacial polymer deposition	≈200	Thermosensitive gel (Pluronic F-127:Pluronic F-68, 30:0.7)	–	[15]
PVP-EC and PVP-Eudragit® RSPO	Acyclovir/SV infection	Nanoprecipitation	≈100	In situ gelling system: 15% Pluronic F-127	–	[16]

EC, ethyl cellulose; *HEC*, hydroxyethylcellulose; *HIV*, human immunodeficiency virus type 1; *HSV*, herpes simplex virus; *NC*, nanocapsule; *NPs*, nanoparticles; *NS*, nanosphere; *PCS*, protease cleavage site; *PDGFR-β*, platelet derived growth factor receptor-β; *PEG*, poly(ethylene glycol); *PEI*, polyethylenimine; *PLA-HPG*, poly(lactic acid)-hyperbranched polyglycerols; *PLGA*, poly(lactic-*co*-glycolic acid); *PVP*, poly(vinylpyrrolidone); *siRNA*, small interfering RNA; *SLNs*, solid lipid nanoparticles.

was observed when the NP size decreased from 900 to 188 nm. Overall and considering factors of encapsulation efficiency and mucoadhesive ability, larger-sized NPs were proved the most efficient. Similarly, Alukda et al. prepared solid lipid nanoparticles (SLNs) loaded with tenofovir by a modified phase-inversion technique and investigated the effect of bovine serum albumin (BSA) concentration, aqueous phase pH, and lipid amount (Softisan 100) on the particle mean diameter [20].

Surface charge is another important characteristic that may determine the particle cellular uptake, cytotoxicity, and diffusion ability. First works to study NP-CVM interactions demonstrated that nonionic and cationic polymers such as polyethylene glycol (PEG) or polyvinylpyrrolidone (PVP) have the ability to alter the gel-like structure of mucus. It was revealed that PEG (3350 Da) can induce mucin fiber coalescence through hydrogen bond formation resulting in larger pore sizes. Whereas, anionic polymers like PLGA did not affect the mucus characteristics [21]. The impact of changing surface charge on particle behavior was investigated using poly(ε-caprolactone) (PCL) NPs loaded with dapivirine and surface modified to produce either negatively [poloxamer 338 NF, and sodium lauryl sulfate (SLS)] or positively [cetyltrimethylammonium bromide (CTAB)] charge NPs [22, 23]. The PCL-based NPs showed an average diameter between 180 and 200 nm, a high drug loading efficiency, a similar or improved antiviral activity, in comparison with free drug, and were readily taken up by several cell lines. However, positive particles (surface modified by CTAB) were significantly more cytotoxic than negative particles (surface modified by poloxamer, or SLS) [23].

Surface modification of NPs with PEG chains, known as PEGylation, aims at enhancing NP transport/delivery across the vaginal mucosa through producing virus-like "stealth" diffusion. Based on this strategy, the formulation of NPs at a small suitable size and neutral PEGylated surface makes them able to penetrate through the mucus gel avoiding entrapment by size-occlusion or ionic interactions with mucus constituents and ultimately leading to enhanced uptake by the underlining epithelial cells. A study by Tang et al. demonstrated that poly(sebacic acid) (PSA)-PEG diblock copolymer NPs with a diameter ~170 nm were able to diffuse in CVM, which bulk viscosity is ~1800-fold higher than water, at a velocity only 12-fold lower than their diffusivity in pure water [24]. Conversly, PSA or PLGA NPs at a similar size diffused at least 3300-fold slower in CVM than in water [24]. Likewise, PEGylated PLGA NPs (170 nm in diameter) administered intravaginal to mice deposited at significantly higher concentration in the tissue of the reproductive tract (fivefold) up to 6 h postadministration showed remarkably greater diffusion through cervical mucus (3- to 10-fold), in relation to unmodified PLGA NPs [25, 26]. PEG density and MW were shown to influence the diffusion of PEGlated NPs; optimal diffusion conditions were achieved with dense surface coating of low MW PEG moieties (2–5 kDa) [25]. However, a more recent study demonstrated that more effective mucus penetration could be acheived by NPs more densely coated with PEG (up to 40 kDa), regardless of PEG MW, indicating that the

physical conformation of PEG on the NP surface is a key for preventing interactions with mucus [27].

Nanoparticle surface functionalizing strategies with targeting ligands have also been exploited for intravaginal delivery. Chemical moieties like antibodies, such as sperm adhesion molecule 1 antibody for sperm targeting [28] and human-leukocyte antigen to target dendritic cells [29], and heparin to target natural killer and vaginal cells [20] have been employed for surface functionalization of NPs administered intravaginally. While targeting is known to enhance the nanosystem specificity and its toxicity profile, it is anticipated that the direct access to the site of action associated with local administration as in the majority of intravaginal delivery systems may limit the need for a targeting ligand.

3.1.2 Nanoparticle-incorporated vaginal dosage forms

Instead of developing targeted NPs, research has focused on developing carriers that can incorporate the drug-loaded NPs and improve their ease of administration and residence time in the vaginal mucosa. The vaginal mucosal administration of aqueous NP suspensions is limited by extensive leakage of the administered dose and poor retention. It has been shown that over 50%–70% of the administered NP dose leaks out within 30 min, with less than 1%–2% of the total NPs is retained at 24 h [26, 30]. Minimizing leakage may necessitate maintaining animals in an inverted position for 1–10 min [26, 30], making translation of such methods to clinical practice unlikely.

For convenient intravaginal self-administration, drug nanosystems require their incorporation into suitable carrying dosage forms. Thus, a wide variety materials such as hydroxyethylcellulose, Carbopol 974P [13], Pluronic F-127:Pluronic F-68 (30:0.7) [15], and poloxamer [12], have been employed to prepare vaginal gels to serve as delivery platforms for NPs. These NP-gel systems have been found to improve permeation, mucoadhesion, and drug retention, enhance drug chemical, enzymatic, and metabolic stability, sustain drug release, and improve local/systemic biodistribution of the drug [15]. In situ thermosensitive gel formulations can combine the advantages of gels with those of liquids, in particular ease of administration. These formulations remain in liquid state before administration and transform to gel after administration into vaginal cavity. For example, Ramyadevi et al. developed an acyclovir-loaded poly(vinylpyrrolidone) (PVP)-Eudragit RSPO hybrid polymeric NPs (diameter ~ 99 nm, zeta potential ~26.1 mV) in 15% Pluronic F-127 vaginal gel [16]. When administered to female Wistar rats, the NPs in situ gel formulation maintained a therapeutic drug level in plasma for once daily therapy with 10 times reduced dose. Furthermore, the formulation demonstrated twofold increase in the relative bioavailability of the drug compared to pure drug suspension.

The fact that healthy human vaginal acidic pH, which is in the range of 4–5, is neutralized by semen and changes in vaginal disorders, has enabled the use of pH-sensitive

NPs that can retain the drug at normal acidic vaginal pH and rapidly release as response to pH increase due to vaginal conditions. Several studies have reported the development of vaginal NPs based on Eudragit S-100, a FDA-approved anionic copolymer consisted of methacrylic acid and methyl methacrylate [15, 31–33]. Owing to its ability to dissolve at pH 7.0 or higher and remains insoluble at acidic pH, Eudragit S-100 is an ideal polymer for intravaginal nanocarriers. More recently, the concept of pH-sensitive release has been employed to develop pH-responsive interconnected porous polyurethane membrane for smart intravaginal release of NPs [9]. The pH-responsive membrane was fabricated by electrospinning to facilitate the production of thin interconnected porous membranes. The permeability of a polystyrene model NP and a CCR5 *si*RNA-loaded SLN through the electrospun porous membrane was controlled by changing the pH-responsive morphology of the membrane and electrostatic interaction between the membrane and the NPs. NPs release through the polyurethane membrane significantly increased, when pH value was increased from 4.5 to 7 [9].

In addition to incorporating conventional drugs, NPs can be used as a platform for the protection and efficient intravaginal delivery of nucleic material and proteins/peptides. The use of small interfering RNA (*si*RNA) as a gene therapy to combat sexually transmitted infections has recently gained increased interest. In a new exemplar study, *si*RNA-polyethylenimine (PEI)-encapsulated NP for the knockdown of PDGFR-β expression has been fabricated as an approach to prevent/reduce sexually transmitted *chlamydia* infection in women [9]. In vitro treatment with PDGFR-β *si*RNA-PEI-PLGA-PEG NP significantly decreased the intracellular *Chlamydia trachomatis* and extracellular release of *C. trachomatis* by approximately 65% and 67%, respectively. As an example of peptide vaginal delivery, a novel candidate HIV vaccine targeting the 12 viral protease cleavage sites (PCSs) (the PCS vaccine), which consists of 12 20-mer peptides, was incorporated into a biodegradable nanoparticle system formed by chitosan and dextran sulfate [11].

3.2 Liposomes

Liposomes are artificial spherical lipid vesicles formed by self-assembly of phospholipids into at least one bilayer membrane enclosing an aqueous center. Liposomes can be classified into unilamellar (one bilayer) or multilamellar (many concentric bilayers). They can be utilized as drug carriers and can provide controlled release of the loaded drugs. Due to their composition, the liposomal cavity is suitable for incorporating hydrophilic drugs, while the phospholipid bilayer can accommodate hydrophobic drugs (Fig. 2). Depending on the preparation method, many forms of liposomes with a diameter ranging from 20 to 3000 nm can be obtained.

Liposomes have been widely investigated as promising nanocarriers for the management of vaginal infections [34–42], and inflammation [43], thanks to their abilities to

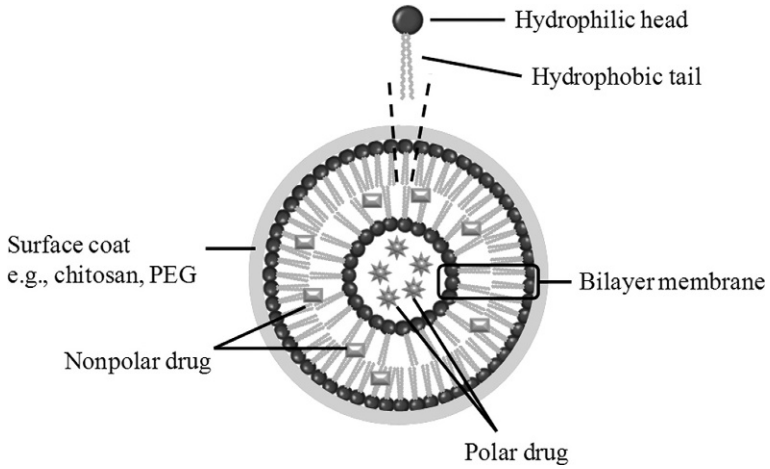

Fig. 2 Schematic representation of a liposomal drug delivery system.

enhance tissue distribution and to control release of incorporated drugs. Examples of liposomal systems for vaginal delivery are described in Table 2.

Anti-infection agents, such as clotrimazole [39, 42], metronidazole [39], and chloramphenicol [41, 42], were the first therapeutic agents to be studied in liposomal intravaginal delivery. These works demonstrated that techniques like polyol dilution and pro-liposome can be used to obtain nano-sized vesicles with a diameter in the range of 200–400 and a good drug loading capacity. In addition, drug entrapment efficiency appeared to be determined by drug physicochemical and affinity characteristics. For example, clotrimazole, which is a hydrophobic drug, showed a remarkable entrapment efficiency up to 95%, whereas less than 10% of metronidazole, which exhibits low water and lipid solubilities, was loaded [39, 42]. In stability studies, the developed liposomes showed good stability at vaginal pH in vitro. Furthermore, in vivo administration of clotrimazole-incorporated vaginal liposomes sustained the drug release resulting in enhanced antifungal activity in the rat model [40].

PEG coating eliminates adhesive interactions between the coated liposome and the vaginal mucus enabling it to penetrate across the mucus, and assuring a close proximity to the vaginal epithelium, which ultimately lead to enhanced drug effectiveness. Lechanteur et al. studied the effect of PEGylation on positively charged lipoplexes loaded with a mix of *si*RNA against two targets implicated in the cancerization induced by HPV16 infection, which are apoptotic protein MCL1 and oncoprotein E7. When tested in HPV16 positive cell lines, PEGylated lipoplexes were found to increase mRNA knockdown by *si*RNA. PEG prevented the toxicity associated with the cationic property of the lipoplex. However, a high percentage of PEG seemed also to reduce *si*RNA endosomal escape, probably by steric hindrance. This limitation was addressed through decreasing PEG

Table 2 Recent examples of liposomes investigated for vaginal drug delivery.

Composition	Active agent/ treatment	Preparation method	Mean size (nm)	Final dosage form	Surface modification	References
Lipoid S 100 (200 mg), CH (10 mg), mPEG 2000 (36.3 mg)	IFN α-2b/HPV infection	Film method	≈180	Suspension	PEG	[34]
DOTAP:CH:DOPE (molar ratio 1:0.75:0.5)	*si*RNA antiapoptotic protein MCL1 and *si*RNA anti-oncoprotein E7/cancer induced by HPV16 infection	Lipid film hydration	≈200–300	Suspension	PEGylation (DSPE-PEG 2000, DSPE-PEG 750 or C8-PEG 2000-ceramide)	[35]
EPC, SDCh, EPG, and PG	Metronidazole or clotrimazole/vaginal infection	Proliposome; polyol dilution, ethanol injection, and dehydration-rehydration	≈180–340	Gel (Carbopol 974P NF, 1% w/w)	–	[36]
Phospholipon 90H, CH, and diacetyl phosphate	Ciclopirox olamine/ fungal infection	Ethanol injection	≈200	Gel (Carbopol 974 P, 2% w/w)	–	[37]
EPC/Ch (5:5 M ratio), EPC:CH:Ole (5:5:2 M ratio), and EPC:CH: sodium Ch sulfate (5:5:2 M ratio)	PEI/HSV infection	Lipid film hydration	≈200	Suspension	–	[38]
SPC	Curcumin/vaginal inflammation	Lipid film hydration	≈200	Suspension	–	[43]

Continued

Table 2 Recent examples of liposomes investigated for vaginal drug delivery—cont'd

Composition	Active agent/ treatment	Preparation method	Mean size (nm)	Final dosage form	Surface modification	References
SPC:DOPE:CH: mPEG-Hz-CHEMS (5:3:1:1 M ratio)	Arctigenin Antioxidant, anti-HIV, antitumor, and anti-inflammatory	Lipid film hydration	≈125	pH and temperature dual-sensitive gel: poloxamer 407 and poloxamer 188	–	[44]
Positively charged (EPC:CH:SA, 2:1:1 M ratio), negatively charged (EPC:CH: DMPG, 2:1:1 M ratio), or neutral (EPC:CH, 2:1 M ratio)	CN54gp140 protein/HIV infection prevention	Dehydration-rehydration	≈120 to 160	Rod-shaped solid dosage form prepared from a lyophilized HEC gel	–	[45]

Ceramide-PEG2000, C8-PEG2000-Ceramide; *CH*, cholesterol; *CS*, sodium cholesteryl sulfate; *DMPG*, dimyristoyl-L-α-phosphatidylglycerol; *DOPE*, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine; *DOTAP*, 1,2-dioleoyl-3-trimethylammonium-propane; *DSPE-PEG2000*: 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[methoxy(polyethylene glycol)-2000]; *DSPE-PEG750*, 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[methoxy(polyethylene glycol)-750]; *EPC*, egg phosphatidylcholine; *EPG*, egg phosphatidylglycerol-sodium; *HEC*, hydroxyethylcellulose; *HPV16*: human papillomavirus 16; *HSV-2*: herpes simplex virus type 2; *mPEG 2000*: methoxy poly (ethylene glycol)-modified lipids; *mPEG-Hz-CHEMS*, methoxy poly(ethylene glycol) 2000-hydrazone-cholesteryl hemisuccinate; *Ole*, oleic acid; *PEI*, polyethylenimine; *PG*, propylene glycol; *SA*, stearylamine; *SDCh*, sodiumdeoxycholate; *SDS*, sodium dodecylsulfate; *siE7*, *si*RNA efficient against the oncoprotein E7; siMCL1 against the antiapoptotic protein MCL-1; *siRNA*, small interfering RNA; *SPC*, soya phosphatidylcholine.

density to 20% using ceramide-PEG2000, which enabled the release of *si*RNA and, in consequence, retain the ability to exert its biological effect [35]. This work highlights the importance of designing the surface coating in such a way to maintain the right balance between cytotoxicity and drug efficacy.

To increase the retention time and assure sufficient drug release at the vaginal site, liposomes have been incorporated into bioadhesive gels such as Carbopol hydrogels [36, 37, 39, 41]. Thermosensitive gel systems composed of a mix of poloxamers can also offer additional advantage of easy administration of the liposomal system [44]. Optimization of both the incorporating gel system and the mechanism of drug release is a key for controlled delivery. Thus, a dual-sensitive liposome gel system that combine a thermosensitive gel, composed of poloxamer 407 and poloxamer 188, and a pH-sensitive liposomes for the vaginal delivery of arctigenin, a natural compound that exhibits antioxidant, anti-HIV, antitumor, and antiinflammatory activities. To create the pH-sensitive property, liposomes were formulated with the addition of the mPEG-Hz-CHEMS polymer, which comprises hydrazone pH-sensitive groups. Arctigenin incorporated into the dual-sensitive liposome gel system showed more stability and less toxicity than drug incorporated in pH-sensitive liposomes suspension. Furthermore, a desirable sustained drug release was observed at pH 5 over a period of 3 days, compared to negligible release at pH 7.4 and 9.0.

3.3 Nanoemulsions

Nanoemulsions, also known as microemulsions, are submicron sized, typically between 20 and 200 nm, colloidal dispersions that are considered thermodynamically and kinetically stable. They are comprised of two immiscible liquids such as oil and water stabilized by an interfacial film composed of a suitable surfactant and a cosurfactant (Fig. 3). Nanoemulsions used as intravaginal transport system that brings about several advantages

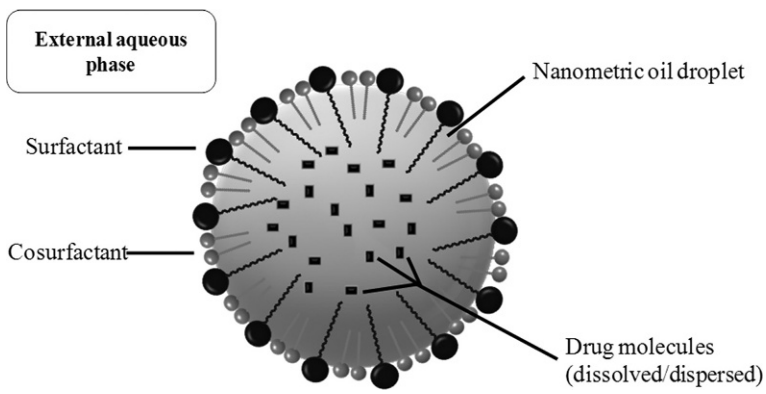

Fig. 3 Schematic representation of an o/w emulsion-based drug delivery system.

including ease of preparation, long-term stability, effective solubilization of lipophilic drugs, optical transparency, protection against oxidation and enzymatic hydrolysis in o/w nanoemulsions, enhanced drug release owing to the large surface area of the nano-sized globules, and improved permeability resulted from surfactant-induced membrane fluidity. The proper formulation of nanoemulsion requires the precise selection of suitable of oil phase and surfactant system that takes into account their toxicity and contribution to the stability of nanosystem. Among others, nonionic surfactants are the most widely used in nanoemulsion formulation owing to their relatively low toxicity, compatibility with ionic active ingredients and other formula components, and high stability over a broad range of pH values.

In general, nanoemulsion-based formulations that have been investigated for intravaginal delivery are of the o/w type and incorporate antiinfective and spermicidal agents [46–50]. Gelling agents, such as Carbopol 940 (1% w/v) [50], chitosan [49], Carbopol ETD 2020 (0.8%, w/w) [47], SeaSpen PF (0.9%, w/w), and Viscarin GP-209 (0.9%, w/w) carrageenans [46], have been included to produce nanoemulsion-based gels for improved viscosity and bioadhesive properties. A gel system comprising Carbopol 934 (0.3%, w/v) and poloxamer 407 (20%, w/v) has also been used in the formulation of a thermosensitive nanoemulsion-based gel [48]. Detailed examples of nanoemulsions used for vaginal drug delivery are given in Table 3.

The mixture of the surfactant and the cosurfactant should be carefully selected to accomplish a stable nanoemulsion formulation. For o/w emulsions, the hydrophilic lipophilic balance (HLB) value should be greater than 10. Ternary phase diagrams can be constructed to identify nanoemulsion regions [48]. Thermodynamically stable nanoemulsions normally require a high percentage of surfactants, which evident in some studies by using up to 35% [48] and 43.5% (w/w) [47] of the surfactant mix.

3.4 Dendrimers

Dendrimers are nano-sized, hyperbranched, *star*-shaped polymers with well-defined size and structure consisting of tree-like arms or branches. They can be constructed with ideal monodispersity and predictable physicochemical properties. According to the structure and number of layers (generations), dendrimers measure between 1.5 and 15 nm in diameter [51]. The unique properties of dendrimers make them suitable carriers for therapeutic actives, which can be either physically accommodated in the void spaces of the dendrimer's interior or chemically conjugated to the surface of the macromolecule (Fig. 4). Furthermore, the surface chemistry of the dendrimer allows efficient functionalization with a broad array of terminal functional groups and targeting ligands.

Dendrimers with macrophage targeting ability have been explored as a strategy in the treatment of HIV infection (Table 4). The rationale behind this is that human vaginal macrophages are known to be infected by HIV and can thus support the spread of the

Table 3 Examples of o/w nanoemulsions used for vaginal drug delivery.

Composition	Active agent/ treatment	Mean size (nm)	Final dosage form	References
Oil phase: Phospholipon 90G (5.1%, w/w) and Captex 300 (10.8%, w/w). Surfactant: Cremophor EL (7.6%, w/w). Co-surfactants: PG (4.2%, w/w) and PEG 200 (4.2%, w/w)	WHI-07 and VDDTC/ prophylactic anti-HIV microbicide	≈30–80	Nanoemulsion-based gel: SeaSpen PF (0.9%, w/w) and Viscarin GP-209 carrageenans (0.9%, w/w)	[46]
Oil phase: Capryol 90 (14%, w/w). Surfactant: Cremophor EL (43.5%, w/w)	Clotrimazole/ vaginal fungal infections	≈50	Nanoemulsion-based gel: optimal gel formulated with Carbopol ETD 2020 (0.8%, w/w)	[47]
Oil phase: tea tree oil (10%, w/w). Surfactant: Tween 20 (23.33, w/w). Co-surfactants: Labrasol (11.67, w/w)	Itraconazole and tea tree oil/vaginal fungal infections	≈40	Thermosensitive nanoemulsion-based gel: Carbopol 934 (0.3%, w/v) and poloxamer 407 (20%, w/*v*)	[48]
Oil phase: soybean oil (13.09%, w/w). Surfactant: Tween 80 (7.44%, w/w). Co-surfactants: PG (8.52%, w/w)	Polyphenon and curcumin/ vaginal bacterial infections	≈210	Nanoemulsion-based gel: chitosan (1% w/w)	[49]
Oil phase: Mentha essential oil (2%, v/v). Surfactant: Tween 80 (7%, v/v). Co-surfactants: PEG 400 (3%, v/v)	Mentha essential oil/ vaginal fungal infections	≈180	Nanoemulsion-based gel: Carbopol 940 (1% w/v)	[50]

HIV, human immunodeficiency virus; *o/w*, oil-in-water; *PEG*, poly(ethylene glycol); *PG*, propylene glycol; *VDDTC*, vanadocene dithiocarbamate; *WHI-07*, 5-bromo-6-methoxy-5,6-dihydro-3′-azidothymidine-5′-(*p*-bromophenyl)-methoxy alaninyl phosphate.

viral infection. Lamivudine-loaded mannosylated [62] and efavirenz-loaded tuftsin-conjugated [63] fifth-generation PPI dendrimers were constructed and evaluated in vitro. The surface decoration of the dendrimers equip them with various advantage leading to increased cellular uptake, minimized toxicity, sustained drug release, and improved anti-HIV activity as compared to nonconjugated dendrimers. In a study to

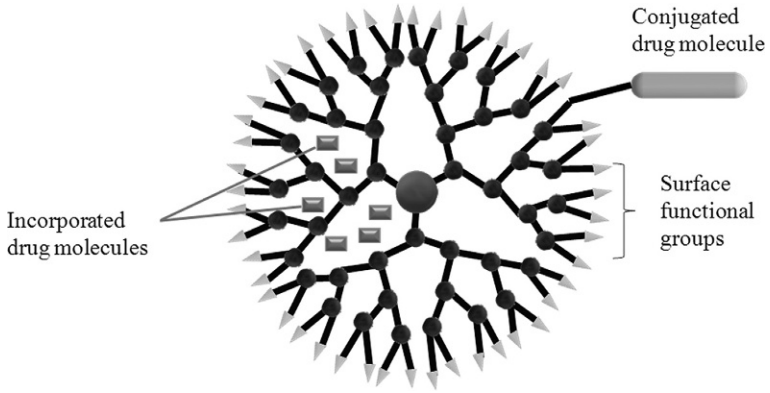

Fig. 4 Structure of a dendrimer-based drug delivery system.

Table 4 Examples of dendrimers developed for vaginal administration.

Generation	Composition	Active agent/ treatment	Final dosage form	Surface modification	References
G1, G2, G3	Polyanionic carbosilane dendrimers	Dendrimer with inherent anti-infective activity/ HIV-1 prophylaxis (microbicide)	Solution	–	[52–58]
G2, G3	Polyanionic carbosilane dendrimers	Dendrimer with inherent anti-infective activity/ HIV-2 prophylaxis (microbicide)	Solution	–	[59]
G1, G2, G3	Polyanionic carbosilane dendrimers	Dendrimer with inherent anti-infective activity/ HSV-2 prophylaxis	Solution	–	[60]
G4	PAMAM	Dendrimer with inherent anti-infective activity/*Escherichia coli* infections/ antimicrobial activity	Solution	–	[61]
G5	PPI	Lamivudine/HIV infection	Solution	D(+)Mannose	[45]
G5	PPI	Efavirenz/HIV infection	Solution	Tuftsin	[62]

HIV, human immunodeficiency virus; *HSV*, herpes simplex virus; *PAMAM*, poly(amidoamine); *PPI*, poly(propylene imine); *SHIV*, simian/human immunodeficiency virus.

evaluate the safety for dendrimeric nanocarrier use in pregnant women, FITC-labeled poly(amidoamine) (PAMAM) dendrimers (~16 kDa) were demonstrated to have slower rate of transport across human fetal membrane (<3% over 5 h) as compared to free fluorescein, within comparison to (49% over 5 h) [64]. Furthermore, the permeability of the dendrimer (5.8×10^{-8} cm^2/s) through the fetal membrane was sevenfold lower than that of FITC (7.9×10^{-7} cm^2/s). These interesting results may indicate that absorption of drugs incorporated/coupled to dendrimers would be restricted across the human fetal membranes enabling these nanosystems to be selectively employed for local intravaginal application to pregnant women.

In addition, a number dendrimers with inherent microbicide activity have emerged as promising anti-HIV-1 candidate agents, which have the ability to interact and inactivate HIV-1, and/or compete with HIV-1 for host cell targets, imparted by the dendrimer's surface functional groups (Table 4). First-, second-, and third-generation polyanionic carbosilane dendrimers have also been synthesized and investigated as candidates against sexually transmitted diseases. These dendrimers have been shown to inhibit HIV-1 activity at an early stage of viral replication and prevent the virus from entry in the host cell by blocking the gp120/CD4/CCR5 interaction. They have also exhibited low cytotoxicity in vitro and high in vivo biocompatibility, and their combinations with tenofovir and maraviroc produced synergistic activity, thus making them very promising bioactives against HIV-1 infection as well as other viruses, such as HSV-2 [52–60]. In addition, bactericidal effects were demonstrated by generation-4 of hydroxyl, carboxylic acid, and amine-terminated PAMAM dendrimers, which showed ability to treat *Escherichia coli* infections in vivo in pregnant guinea pigs on topical cervical administration [61]. Antibacterial mechanisms suggested included electrostatic binding (NH_2 terminated) or hydrogen bonds (OH-terminated) with components of the *E. coli* membrane or chelating the divalent ions (COOH-terminated) in this membrane.

3.5 Cyclodextrins

Cyclodextrins (CDs) are cyclic oligosaccharides consisting of 6 (α-cyclodextrin), 7 (β-cyclodextrin), 8 (γ-cyclodextrin), or more D-(+)glucopyranose units linked by α-(1,4) glucosidic bonds. They are produced by degradation of starch by cyclodextrin glucanotransferase enzyme and measure around 1 nm in diameter. Due to their distinctive structure, CDs can form solid inclusion complexes, also known as host-guest complexes. In these complexes, a guest molecule, or its hydrophobic region, is included in lipophilic internal cavity of the host CD molecule and, hence, solubilized by the hydrophilic outer surface (Fig. 5) [64].

This feature has been widely exploited to formulate water-soluble inclusion nanocomplexes of cyclodextrins with hydrophobic drugs intended for vaginal use, including female sex hormones, e.g., progesterone [65], anti-*Trichomonas vaginalis* parasitic

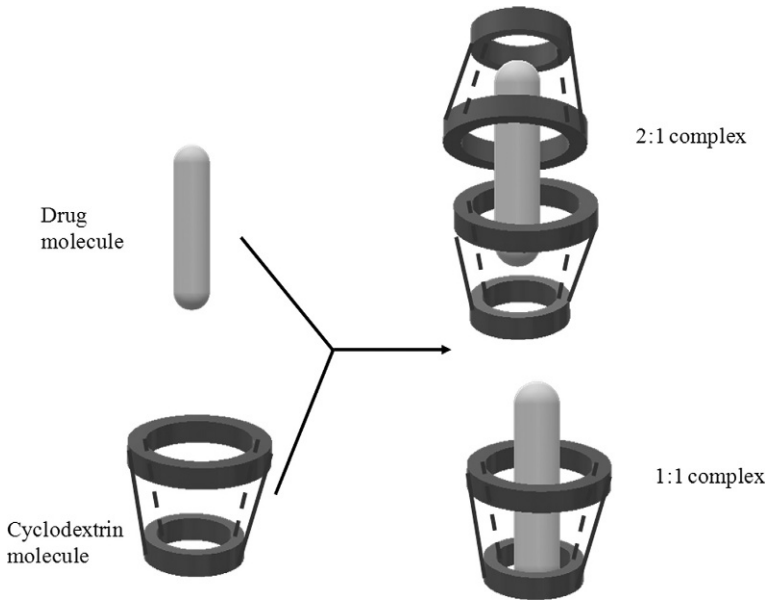

Fig. 5 Schematic representation of drug-cyclodextrin host-guest inclusion complexes.

infection (metronidazole) [66], antifungal agents (such as fluconazole [67], clotrimazole [68], and voriconazole [69]), anti-HIV microbicidal agents (UAMC01398) [70], and anti-cervicitis agents (baicalein) [71] (Table 5).

Residence time in the vaginal cavity can be increased and drug release sustained by incorporating the inclusion complexes into mucoadhesive vaginal formulations. Examples of such formulations include mucoadhesive gels, such as those prepared with HPMC [68] and hydroxypropylmethylcellulose (HPMC), poly(acrylic acid) (PAA) or sodium alginate (SA) [67], mucoadhesive tablets, using Carbopol 934, HPMC, and Xanthan gum 200 [72], and in situ thermosensitive mucoadhesive gels, for example, poloxamer-407 and poloxamer-188 gel system [69].

4. Conclusions

The distinctive physiological characteristics of the vagina and continuous changes in the composition of the vaginal environment impose a challenge for efficient treatment using drugs administrated intravaginally. Besides, conventional vaginal pharmaceutical formulations are associated with various disadvantages that affect patient compliance making drug treatment via the vaginal route even more challenging. To address these drawbacks, nano-sized systems have emerged as promising platforms for vaginal drug delivery. These nanoformulations include nanoparticles, liposomes, nanoemulsions, dendrimers, and cyclodextrins, which in addition to delivering small molecule drugs, macromolecules,

Table 5 Examples of cyclodextrins for vaginal drug delivery.

Cyclodextrin derivative	Active agent/ treatment	Final dosage form	References
Methylated-β-CD	Metronidazole/ *Trichomonas vaginalis* infection	Solution	[66]
β-CD	Fluconazole/ vaginal infections	Hydrogel: HPMC, PAA, SA (various molar ratios)	[67]
HP-β-CD	Clotrimazole/ fungal infections	Hydrogel: HPMC 1% (w/w)	[68]
HP-β-CD	Voriconazole/ fungal infections	Thermosensitive hydrogel: P407, P188, SA, HPMC; molar ratio of optimal formula: 18:5:0.4% (w/w)	[69]
SBE-β-CD	UAMC01398/ HIV infection	Hydrogel: HEC (1.8%, w/w)	[70]
HP-γ-CD	Baicalein/ cervicitis	Thermosensitive hydrogel: P407, P188, SA, HPMC, and benzalkonium bromide; molar ratio of optimal formula: 18:0.96:0.5:4.0:0.02% (w/w)	[71]

CD, cyclodextrin; *HEC*, hydroxyethylcellulose; *HPMC*, hydroxypropylmethylcellulose; *HP-β-CD*, 2-hydroxypropyl-β-cyclodextrin; *HP-γ-CD*, hydroxypropyl-γ-cyclodextrin; *P188*, Poloxamers 188; *P407*, Poloxamers 407; *PAA*, poly (acrylic acid); *SA*, sodium alginate; *SBE-β-CD*, sulfobutyl ether-β-cyclodextrin.

and *si*RNA, have shown in some cases intrinsic therapeutic effects, such as microbiocidal activity of some dendrimers.

Both the core and the surface of a nanosystem can be precisely engineered in such a way to possess the desirable characteristics for successful vaginal drug delivery. Consequently, the nanosystem can be equipped with abilities to be retained in the vaginal cavity, penetrate through mucus to the epithelial mucosa, enable extended extracellular drug release or intracellular drug delivery, and as a result improve treatment efficiency and minimize the frequency of administration.

Vagina drug delivery nanotechnologies have been proved effective in vivo in animals; however, giving the limitations of animal models clinical trials are necessary to confirm the effectiveness, and safety in humans. While the safety issue of nanoformulation has been studied extensively on parenteral, pulmonary, and skin products, there is little information on the toxicity profile of these systems in humans. Certainly, clinical studies can generate more data on the toxicity and biocompatibility of the nanosystems and the interference with the normal functions and environment of the vagina such as mucosa composition, vaginal pH, and healthy vaginal microbiota, especially after repeated administration. The increased feedback coming from clinical use will hopefully help focusing the efforts on designing safer and more effective nanoformulations.

Conflict of interest

The authors state no conflict of interest.

Acknowledgments

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CHAPTER 12

Regenerative nanomedicine applications for neurodegenerative diseases of central nervous system

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1. Introduction

As the brain age, the whole nervous system is affected, and the decline in sensory, motor, and cognitive functions is experienced. However, the aging rate is not affecting individual uniformly, and a considerable variability is observed between individuals and within the individual. There is a higher incidence of a neurodegenerative disease such as Alzheimer's disease, Parkinson's disease, Huntington disease, aged-related macular degeneration, and glaucoma in the sixth, seventh, and eighth decades of life. For a neurodegenerative disease such as amyotrophic lateral sclerosis, the risk of developing the disease rises sharply in individuals over 40 years of age [1].

In the past decades, a large amount of studies have been dedicated to the identification of the genetic and cellular markers of these diseases, but still, the events that determine the selective neuronal vulnerability remain elusive. The current therapies failed to alter the progression of the diseases and mostly ease the associated symptoms.

In recent years, application of nanotechnologies in the field of regenerative medicine has demonstrated some encouraging progress. Nanomedicines were developed to overcome physiological barriers, improve the non-parenteral targeted delivery of drugs, genes, or proteins, ensure a slow release over a long period, minimize toxicity to surrounding tissue, and provide a scaffold to allow cell replacement therapy.

This chapter discusses some major neurodegenerative diseases, and the nanomedicine approaches developed to slow the progression of these neurodegenerative diseases.

2. Central nervous system

The central nervous system (CNS), composed of the brain, spinal cord, and, retina, has a limited capacity to regenerate spontaneously following debilitating diseases or traumatic injuries. The neurodegenerative diseases affect primarily individuals in their mid- to late

Theory and Applications of Nonparenteral Nanomedicines
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life [2]. The incidence is also expected to burgeon in part due to the extension of the lifespan [3]. The neurodegenerative diseases are complex and are associated with the progressive loss of motor, sensory, and/or cognitive functions. Despite the progress made in the understanding of the progression of these diseases, the therapeutic strategies remain limited. New therapeutic approaches are critically needed.

Examples of most common neurodegenerative diseases include Alzheimer's disease, Parkinson's disease, Huntington disease, amyotrophic lateral sclerosis, as well as retinal diseases such as retinitis pigmentosa and age-related macular degeneration and optic nerve pathologies such as glaucoma and acute optic neuropathies. The regions of the brain affected by the neurodegenerative diseases mentioned in this chapter are shown in Fig. 1.

2.1 Alzheimer's disease

Alzheimer's disease (AD) is the most common cause of dementia, and estimates suggest that more than 44 million live with the disease worldwide [4]. This number is expected to soar by more than threefold by 2050 [4]. The disease is prevalent in the older population, accounting for 10%–30% for population over 65 years of age and roughly doubling in prevalence every 5 years [5]. The early stages of AD are symptomatically discrete and can develop over several decades before a diagnosis can be established. At the time of diagnosis, the duration of the illness is from 8 to 10 years [6]. While the majority of AD cases are sporadic, mutations in amyloid precursor protein (APP), presenilin 1 (PSEN1), and presenilin 2 (PSEN 2) have been associated with a familial form of AD which represents less than 1% of all AD cases and developed in younger adults typically between 30 and 50 years of age [7]. AD is a multifactorial chronic illness that affects broad areas of the cerebral cortex and hippocampus [6]. The disease slowly progresses from the frontal and temporal lobes to other areas of the neocortex at a rate which is highly variable between individuals [6]. The loss in recent memory is a common characteristic of aging and AD [8]. AD is driven by the extracellular deposition of amyloid-β peptides leading to the formation of plaques, and intracellular accumulation of phosphorylated tau protein forming neuritic (senile) plaques, neurofibrillary tangles, and neuropil threads [9]. As the disease progresses, their quantity, size, and distribution increase through the brain [10]. The amyloid-β plaques promote synapse degeneration and loss of neurons [11]. The cholinergic neurons in the basal forebrain are the first neurons affected in early AD, and selectively degenerate as the disease progresses [12]. The choline acetyltransferase enzyme and acetylcholinesterase are found lowered in all AD patients after postmortem examination [13]. Intuitively, stopping the degradation of these neurons and replacing the lost neurons are potential therapeutic strategies for AD. The current treatments for AD provide symptomatic relief for some patients but do not slow down the progression of the disease. Cholinesterase inhibitors are the only approved treatment

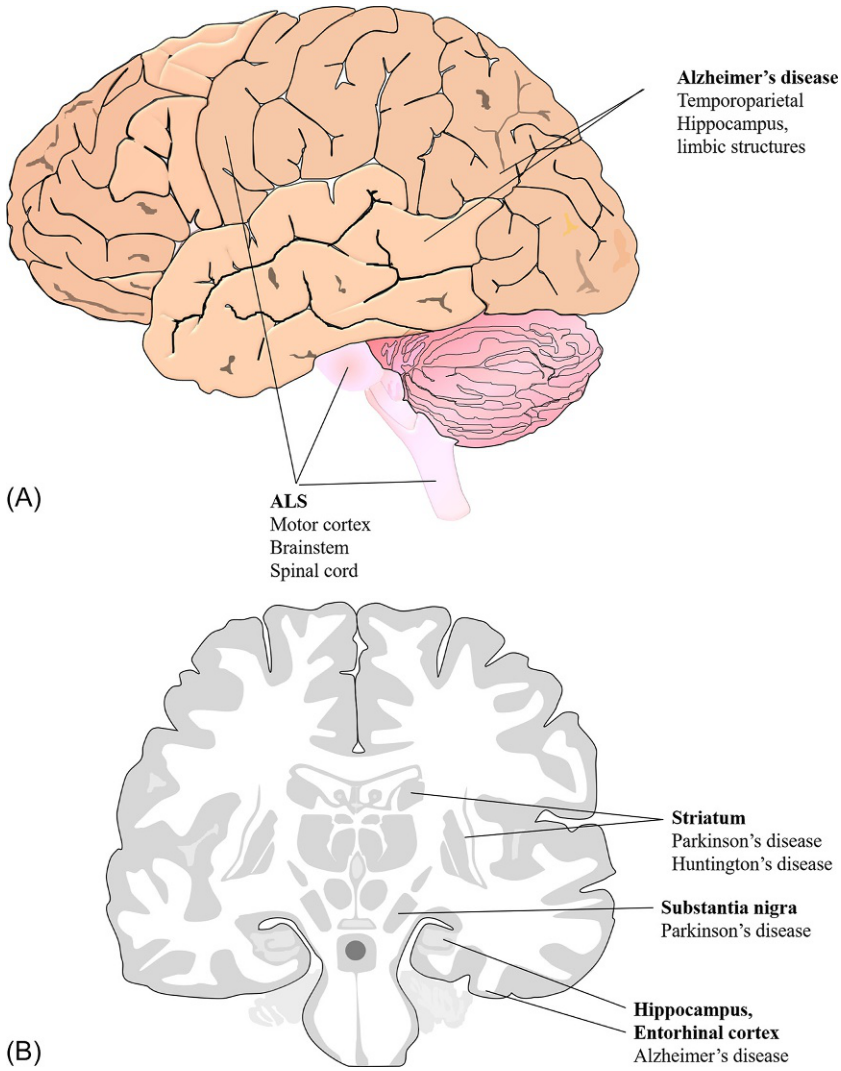

Fig. 1 Sites of development of neurodegenerative diseases in lateral (A) and axial (B) views of the brain.

for the mild-to-moderate AD patients and allow some patients to delay the decline and perform daily living tasks [14]. Memantine is an *N*-methyl-D-aspartate antagonist approved by the FDA to treat moderate-to-severe AD and may improve the function of hippocampus neurons and provide symptomatic relief [15]. Additional drugs are currently evaluated in multiple clinical trials [16].

In recent years, several studies have developed nanomedicines or nanomaterials for the delivery of drugs or for promoting tissue regeneration [17]. One of the most

significant issues for the delivery of nanocarriers to the neurodegenerative area of the brain is to bypass or lure the blood-brain barrier (BBB). The development of AD disrupts the integrity of the BBB, and the disruption is a critical factor for its development; but the extent of the BBB alterations remains controversial as more studies are required [18]. Several preclinical strategies have been developed to achieve brain delivery of the nanocarriers by using surfactants, or by targeting specific endothelial receptors such as low-density lipoprotein receptor (LDLR), transferrin receptor, epidermal growth factor receptor (EGFR), and insulin growth factor receptor (IGFR), or using alternative route of delivery such as intranasal [19]. The surfactants such as polysorbate 80 and poloxamer 188 have increased the delivery of drug-loaded nanocarriers to the brain, by absorbing apolipoprotein A-I and/or apolipoprotein E from the blood, which facilitate the BBB crossing by transcytosis [20]. However, the surfactant coating strategy lacks specificity and can promote drug delivery to multiple other organs [19]. Several targeted nanocarriers were developed to specifically target the amyloid-β peptide (for review, see Ref. [21]). The intranasal route of delivery of drugs to the brain is promising as it allows to bypass the BBB, avoids the first-pass metabolism, improves pharmacokinetic, reduces the side effects associated with systemic drug delivery, and enhances compliance of the patients [22]. Several nanocarriers encapsulating drugs for the improvement in AD symptoms were assessed using intranasal route. Fazil et al. developed a rivastigmine chitosan nanoparticle for intranasal drug delivery and demonstrated higher transport efficiency of the acetylcholinesterase inhibitor (AChEI) through the nasal route compared to the intravenous one in Wistar rats [23]. Arumugam et al. used a liposomal formulation to deliver rivastigmine through the nasal route to the brain and demonstrated higher drug delivery when compared to the free drug or through the oral route in Wistar rats [24]. Yang et al. engineered a liposome harboring on its surface a cell-penetrating peptide to deliver rivastigmine through the nasal route [25]. Rivastigmine concentration increased in the hippocampus, cortex, and olfactory region following intranasal delivery without prominent toxicity on the nasal mucosa in Wistar rats [25]. Jogani et al. developed a tacrine microemulsion and observed higher uptake of AChEI after intranasal administration in the brain of rabbits compared to intravenous injection [26].

Other strategies used growth factor-loaded nanocarriers to slow the progression of the disease and also to promote neuronal regeneration [27]. Several studies have reported the promising therapeutic effects of growth factors on different AD biomarkers [28–30]. Few growth factors known to promote neurogenesis have been used for regenerative brain studies such as the brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), glial-derived neurotrophic factor (GDNF), and basic fibroblast growth factor (bFGF) [31]. The encapsulation of these factors into nano- or microcarriers has improved the growth factor stability and delivery to the brain by overcoming the BBB [32, 33].

Péan et al. demonstrated that the intraseptal implantation of NGF-loaded PLGA microspheres increased the number of acetylcholinesterase (AChE)-positive neurons

by 60% compared to 30% in untreated rats in an animal model of AD with transection of the septohippocampal pathway [34] (Table 1). PLGA is a synthetic biocompatible polymer that has demonstrated a high encapsulation efficiency for growth factors (42%–100%) [19]. Gu et al. demonstrated that NGF-loaded PLGA microspheres implanted in the basal forebrain in a rat model of Alzheimer's disease increased the survival of axotomized cholinergic neurons and improved the spatial learning and memory abilities of the animals tested [39] (Table 1). Zhang et al. prepared a basic fibroblast growth factor (bFGF)-loaded pegylated PLGA nanoparticles coated with *Solanum tuberosum* lectin to target the nasal epithelium and bypass the BBB [40]. The intranasal administration demonstrated a significant improvement in spatial learning and memory in a rat model of AD (Table 1) [40].

Table 1 Neurotrophin encapsulated nano- and microcarriers for the treatment of CNS diseases.

Neurotrophin	Disease model	Carrier	Animal study	Reference
GDNF	Parkinson disease	PLGA microspheres	Intracerebral injection. Protect the dopamine neurons during 6 weeks, when injected at the same time as the 6-hydroxydopamine-induced Parkinson's disease	[35]
GDNF	Parkinson's disease	Liposome	Intranasal delivery. GDNF brain levels increased within 1 h following a single 50 μg dose and returned to baseline by 24 h	[36]
GDNF	Parkinson disease	Cationic liposome	Intranasal delivery. Higher number of tyrosine hydroxylase (TH)-positive neurons in 6-hydroxydopamine model of Parkinson's disease	[37]
GDNF	Parkinson disease	Chitosan (CS)-coated nanostructured lipid carriers, with the surface modified with transactivator of transcription (TAT) peptide.	Intranasal delivery. Motor recovery and higher number of tyrosine hydroxylase fibers and neurons in the striatum and substantia nigra, respectively	[38]

Continued

Table 1 Neurotrophin encapsulated nano- and microcarriers for the treatment of CNS diseases—cont'd

Neurotrophin	Disease model	Carrier	Animal study	Reference
NGF	Alzheimer disease	PLGA microspheres	Basal forebrain implantation. Unilateral fimbria-fornix lesion in rat. Improve the ability of spatial learning and memory in rats with fimbria-fornix lesion	[39]
NGF	Alzheimer disease	PLGA microspheres	Stereotaxic implantation in the septal area. Rescue cholinergic neurons at 2 and 6 weeks after axotomy of the septohippocampal neurons	[34]
bFGF	Alzheimer's disease	Lectins modified PEG-PLGA nanoparticles	Intranasal delivery. Improvement of spatial learning and memory compared to AD control group	[40]
bFGF	Retinitis pigmentosa	Gelatin nanoparticles	Intravitreal injection. Inhibit photoreceptor apoptosis.	[41]
GDNF	Retinitis Pigmentosa	PLGA microspheres	Intravitreal injection in rd1/rd1 mice. Delay of rod photoreceptors degeneration.	[42]
GDNF/melatonin	Retinitis pigmentosa	PLGA/Vitamin E microspheres	Intravitreal injection. Rescue of the photoreceptors in *rho* (−/−) mice after 12 weeks.	[43]
GDNF	Glaucoma	PLGA/Vitamin E microspheres	Intravitreal injection in rabbit. Sustained controlled release of GDNF for up to 6 months.	[44]
GDNF	Glaucoma	PLGA/Vitamin E microspheres	Intravitreal injection. Increased RGC survival observed after 7 weeks	[45]
GDNF	Glaucoma	PLGA microspheres	Intravitreal injection. RGC survival and axon survival in 10% of the treated rats.	[46]
GDNF	Glaucoma	PLGA microspheres	Intravitreal injection in DBA/2J Mouse. 12.6% increase in RGC density in 8 months Increased survival of RGC.	[47]
NGF, BDNF	Glaucoma	Magnetic nanoparticles	Intravitreal injection in zebrafish. Prevention retinal ganglion cell loss induced by oxidative stress	[48]

Table 1 Neurotrophin encapsulated nano- and microcarriers for the treatment of CNS diseases—cont'd

Neurotrophin	Disease model	Carrier	Animal study	Reference
Gene delivery				
GDNF	Parkinson disease	CK30PEG10k (30-mer lysine peptide covalently linked to 10 kDa polyethylene glycol)	Intranasal delivery. Long-term expression for up to 6 months	[49, 50]
GDNF	Parkinson disease	CK30PEG10k	Intracerebral (striatum and/or substantia nigra). Dopamine (DA) neurons and fiber terminals in greater number, and better recovery of motor function	[51]
GDNF	Retinitis pigmentosa	Pegylated peptide for ocular delivery (CGGG (ARKKAAKA)4)	Injections into subretinal space. Physiological rescue of blue light induced retinal degeneration up to 37 days postinjection	[52]
GDNF	Age-related macular degeneration and retinitis pigmentosa	Pegylated peptide for Ocular delivery (CGGG (ARKKAAKA)4)	Injections into subretinal space. Greater functional response of the eye (27%–39%) 7 days post-blue light induced retinal degeneration	[53]

The current treatments of AD are more focused on the relief of the symptoms rather than the inhibition or reconstruction of the affected areas. Blurton-Jones showed that the transplantation of neural stem cells rescues the cognitive phenotype in a transgenic mouse model of AD [54]. The NSCs promoted the secretion of BDNF, leading to an increased number of synapses and restoring hippocampal-dependent cognitions [54]. A similar observation was made by Zhang et al., where NSC transplantation recovered the memory loss phenotype in a transgenic mouse model of AD [55]. Chen et al. reported that a combination of NSCs and NGF-loaded PEG-PLGA nanoparticles implanted into the hippocampal and basal forebrain significantly increased the number of basal forebrain cholinergic neurons, functional hippocampal synapses, and AchE-positive fibers which improved learning and memory functions in a rat model of AD [56]. More recently, the use of nanofiber scaffold to promote cell adhesion and guidance was shown to improve spatial learning and memory in a rat model of AD. Cui et al. used a self-assembling peptide, RAD16, consisting of alternating hydrophilic and hydrophobic

amino acids forming a nanofiber scaffold; the scaffold was functionalized Tyr-Ile-Gly-Ser-Arg, a laminin-derived motif, to promote cell adhesion [57]. The transplantation of the NSCs with the nanofiber scaffold improved spatial learning and memory deficits in AD rats [57].

2.2 Parkinson's disease

The pathological hallmark of Parkinson's disease (PD) is the degeneration of the nigrostriatal dopaminergic neurons and the development of motor deficit disorders associated with the deficit in dopamine regulation [58]. PD is characterized by three cardinal signs such as tremor, rigidity, and bradykinesia [59]. The prevalence of PD is increasing as the population is aging and represents 1% of the people over 65 years of age worldwide and increases up to 4% in older population [60]. Early signs of the disease in younger adult before the age of 40 is only seen in 5% of the cases in population-based cohorts [59]. PD is considered a sporadic disease as the cause remains unknown in the majority of cases. However, familial genetic predisposition factors have been identified that increase the risk to develop the disease by two to threefold for first-degree family affiliation [59]. The familial forms of PD account for 5%–10% of all PD cases and are associated with mutations in leucine-rich repeat kinase 2 (LRRK2), Parkinson's disease protein 2 (PARK2), Parkinson's disease protein 7 (PARK7), PTEN-induced kinase 1 (PINK1), ATPase type 13A2 (ATP13A2), alpha-synuclein (SNCA), or other unidentified genes [61, 62]. The clustered nature of PD, where multiple genes and their interaction with the environment could be potential contributors, cannot be explained by the alteration of a single predisposition gene [62]. PD is characterized by the presence of Lewy bodies made by the intraneural accumulation of α-synuclein and ubiquitin [63]. The accumulation of α-synuclein disrupts the protein trafficking between the endoplasmic reticulum and Golgi [64], and alters the transport of endosomes and autophagosomes [65]. The impaired vesicle trafficking promotes the accumulation of damaged proteins and organelles and ultimately leads to cell death [65]. The localization and distribution of the Lewy bodies seem to follow a specific pattern indicative of the progression of the disease and are found in the substantia nigra when the disease becomes more advanced [63, 66]. However, the implication of the α-synuclein aggregates within the Lewy bodies as the cause or a surrogate marker of the neurodegeneration in PD is challenged by the neuronal degeneration preceding the development of Lewy body in the substantia nigra [67] and the density of the Lewy bodies does not correlate with the nigral neuron losses [68]. Small α-synuclein aggregates or assemblies were shown to affect neuronal cell viability and their excretion impaired neighboring cells [69].

The current treatments for PD are focused on the relief of the symptoms and neuroprotective effects. Dopamine replacement therapy, using the dopamine precursor levodopa (L-3,4-dihydroxyphenylalanine or L-DOPA), is eventually prescribed to all PD patients to ease the symptoms [70]. Levodopa can cross the BBB and is usually

given in combination with a dopamine decarboxylase inhibitor such as carbidopa or benserazide to prevent metabolism of levodopa outside the brain and consequently ensuring that a sufficient amount of dopamine reaches the brain [71, 72]. However, the efficacy of levodopa decreases as the PD progresses, as there is no evidence supporting the theory that this therapy delays dopaminergic neuron degradation [63]. Several strategies have been developed to regenerate the loss of dopaminergic neurons. Early preclinical studies using human fetal ventral mesencephalon (hFVM) graft implantation in the brain of rats with complete lesions of the nigrostriatal DA pathways improved certain aspects of their motor and sensory behavior [73, 74]. The grafting of hFVM in patients demonstrated promising results but highlighted the requirement for renewable biological source [75].

In recent years, nanomedicines have been assessed in preclinical studies to target several pathological features of PD such as α-synuclein aggregates, oxidative stress, mitochondrial dysfunction, and inflammation [76]. These polymeric nanoparticles, phospholipid nanoparticles, solid nanoparticles, or nanoemulsions injected in the systemic circulation demonstrated neuroprotective effects in PD animal models [76]. Gambaryan et al. encapsulated levodopa in PLGA nanoparticles to improve drug delivery to the brain through the intranasal route in Wistar rats with 6-hydroxydopamine-induced PD [77]. The intranasal delivery of levodopa nanoparticles increased the level of dopamine in the rat brains compared to animals treated with levodopa alone or in combination with benserazide [77]. Furthermore, treatment with the levodopa nanoparticles improved the movement performance for a more extended period when compared with the other treatments in this study [77].

Neurotrophic factors have exhibited regenerative abilities in various neurodegenerative diseases, including AD, as mentioned previously (Table 1). As the neurotrophic factors do not significantly penetrate the BBB from the circulation and have a short half-life, strategies were developed to ensure the delivery to the affected areas of the brain [78]. Several studies have encapsulated GDNF or its coding sequence and assessed the neuroprotective effect and regenerative properties in animal models of PD using nonparenteral delivery. In an early study, Jollivet et al. encapsulated GDNF into PLGA microspheres and implanted them into the striatum 2 weeks after the injections of the neurotoxin 6-hydroxydopamine into the same rat brain area to induce PD-like symptoms [35]. The 6-hydroxydopamine injections led to the progressive degeneration of dopaminergic neurons [35]. The infusion of GDNF in denervated striatum was previously shown to promote sprouting of the remaining dopaminergic fibers [79–82]. The encapsulation of GDNF into PLGA allowed a sustained release of the neurotrophic factor for 49 days. The GDNF-PLGA microsphere treatment increased the number of dopaminergic innervation in the striatum but not the number of dopaminergic neurons in the substantia nigra [35]. The behavior of these animals has also statistically improved by the GDNF-PLGA microsphere treatment. Besides, no toxicity was observed as a result of the biocompatibility of PLGA and the slow release of GDNF from the

microspheres [35]. Migliore et al. demonstrated that GDNF encapsulated into cationic liposomes administrated by the intranasal route and given 1 h before stereotaxic injection of 6-hydroxydopamine in rat brain was sufficient to increase the number of tyrosine-hydrolase positive neurons, a marker of dopaminergic neurons in the substantia nigra [37] (Table 1). The GDNF-liposome intranasal delivery induced a neurotrophic effect and protected the dopaminergic neurons in the substantia nigra [37]. Bender et al. showed that liposomal delivery of GDNF through the intranasal route increased the concentration of GDNF in the targeted area within the physiological range to promote neuroprotection and recovery of dopaminergic neurons [36] (Table 1). Hernando et al. encapsulated GDNF into chitosan-coated lipid nanocarriers, modified on their surface with the transactivator of transcription (TAT), a cell-penetrating peptide, compatible with the intranasal route for drug delivery to the brain [38]. The TAT peptide was shown to enhance drug delivery to the central nervous system through the intranasal route [83]. The GNDF-TAT-nanocarrier was given through the intranasal route to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of PD [38]. The treatment was initiated at the same time as the neurotoxin MPTP administered over 3 weeks. The delivery of GDNF improved the behavior of the animals and increased the number of tyrosine hydroxylase positive fibers in the striatum and neuron in the substantia nigra [38]. Harmon et al. encapsulated GDNF expression plasmid into PEGylated poly-lysine nanoparticles [50]. The rats were given the GDNF plasmid nanoparticles through the intranasal route 7 days before unilateral injection of 6-hydroxydopamine to induce the degeneration of the dopaminergic neurons [50]. The intranasal delivery of GDNF plasmid was sufficient to reduce the lesion severity in the substantia nigra as measured by the density of tyrosine hydrolase by immunohistochemistry 3 weeks after the lesions were created [50]. The same group later demonstrated that dopaminergic fiber density and cell counts in the substantia nigra and nerve terminal density in the lesioned striatum were significantly preserved in rats pretreated with intranasal GDNF plasmid [49] (Table 1). Yurek et al. injected GDNF plasmid nanoparticles into the substantia nigra 1 week before treatment with 6-hydroxydopamine and also demonstrated that the level of expression of the neurotrophic factor was sufficient to minimize the effect of the neurotoxin on the neurons of the substantia nigra [51].

Altogether, these studies demonstrated the potential of the intranasal route and GDNF protein or GDNF DNA-loaded nanoparticles for the regeneration of brain regions affected by PD.

2.3 Huntington disease

Huntington disease (HT) is a hereditary progressive neurodegenerative disease caused by an autosomal dominant mutation. The prevalence of the disease is 4–10 per 100,000 in the Western societies [84]. HT occurs typically in middle-aged individual, affects

movement, and is characterized by cognitive decline and psychiatric disturbance; death occurs within 15–20 years from the onset [85]. The disease is caused by the increased number of CAG repeat by 40 or more in the huntingtin (HTT) gene, which encodes for the huntingtin protein [86]. The number of CAG repeat is inversely correlated with the age of onset of the disease [87]. A long polyglutamine chain characterizes the mutant protein as the result of the CAG repeats, which causes cellular dysfunction and death [88]. Additional studies have also demonstrated that the RNA encoding the mutated HTT may also be toxic, as well as the result in loss of function of HTT, and may contribute to the pathogenesis [89]. Prominent neuronal loss is observed in the neostriatum (caudate nucleus and putamen) of late-stage HD patients [90]. The medium spiny neurons of the striatum are particularly sensitive to the mutated HTT, and account for nearly 95% of all neurons in the neostriatum [90]. In addition to the striatum, the degeneration is also extended to cerebral neo- and allocortex, thalamus, pallidum, brainstem, and cerebellum regions during the development of HT [90].

HT is dominantly inherited, and predictive testing can inform whether the disease will develop or not; but not when. The diagnosis of HT is made based on signs of chorea, dystonia, bradykinesia, incoordination, motor impersistence, and slow saccadic eye movement [91, 92]. The cognitive impairments affecting executive functions and behavioral symptoms may develop before the motor symptoms [92].

Currently, there is no treatment for HD; all the developed approaches are targeting the symptoms such as movement, cognitive, and psychiatric disorders to improve the patient quality of life without blocking or slowing the progression of the disease [85, 90].

The early preclinical studies on neuronal regeneration for the treatment of HT relied on allograft or xenograft implantation in the striatum. Several studies have demonstrated that cell suspensions, prepared from the whole ganglionic eminence (WGE) obtained from rat embryos E15, transplanted into the unilateral striatum were sufficient to inhibit the deteriorating motor function. Further studies using graft tissue or cells derived from human tissue demonstrated their efficacy in various models of HT (for review see Ref. [93]). Transplantation therapies were also conducted in HT patients, tissue fragments of the whole ganglionic eminence obtained from fetuses were implanted at multiple sites in the caudate and putamen. These studies demonstrated that tissue transplantation was safe and the positive effect lasted for more than 4–6 years following transplantation but raised ethical issues regarding the source of the embryonic tissues (for review see Ref. [93]). The transplantation of stem cells not only appeared as an alternative to this ethical conundrum and reported functional restoration in animal models but also displayed large variabilities in the assessment methodology and insight for further studies (for review see Ref. [93]).

The striatum of HT patients displays a significant loss of BDNF protein, which likely plays a role in the etiology of the disease [94]. The administration of exogenous neurotrophic factors in animal models of HT demonstrated neuroprotective effect, NGF,

BDNF, GDNF, and ciliary neurotrophic factor (CNTF) exhibited potent neuroprotective effects on several neuronal populations of the striatum [95–97]. Menei et al. demonstrated that the intrastriatal implantation of PLGA microsphere loaded with NGF reduced the striatal lesion induced by quinolinic acid in a rat model of HT [97]. Animal model of HT can be generated by the infusion of excitatory neurotransmitter such as quinolinic acid [98, 99]. Particles producing CNTF encapsulated into poly(acrylonitrile-co-vinyl chloride) were implanted into the lateral ventricle 8–12 days before quinolinic acid injections into the rat striatum. These particles protected cholinergic and GABAergic neurons and improved behavior [100, 101].

Alternative strategy targeted the oxidative stress associated with neuronal degeneration; Bhatt et al. used rosmarinic acid-loaded solid lipid nanoparticles to achieve neuronal protection in rat models of Huntington disease [102]. Rosmarinic acid was previously shown to have neuroprotective effects [103]. The intranasal delivery of rosmarinic acid-loaded solid lipid nanoparticles was able to rescue locomotion and motor coordination as well as decrease the striatal oxidative stress in rat HD model induced by 3NP [102]. Godinho et al. used β-cyclodextrins nanoparticles to encapsulate HTT siRNA; the nanoparticles were implanted in the striatum of R6/2 mice, a model of HD [104]. The HTT protein expression was decreased in the striatum up to 7 days post-injection, and repeated injections were able to restore some behavioral aspects such as balance and motor coordination but failed to affect spontaneous locomotor activity, grip strength, and clasping behavior [104].

The treatment of HD has so far limited success, future studies combining neurotrophic factor delivery using nano- or microparticles and stem cells implantation in the striatum may provide a better understanding of the regenerative process associated with HD.

2.4 Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS), or Lou Gehrig disease is a progressive, lethal disease targeting the upper and lower motor neurons primarily. The death occurs after respiratory paralysis within 3–5 years following the symptomatic onset [105]. The molecular mechanisms at the origin of the disease are mostly unknown, but recent studies have implicated neuroinflammatory processes [106]. ALS affects 1/100,000 adults throughout the world, with 90%–95% cases of sporadic [107]. ALS is a multifactorial disease with mutations identified in more than 30 genes, but mutations in superoxide dismutase 1 (SOD1), transactive response DNA-binding protein (TARDBP), FUS, and C9ORF72 are observed more frequently in the sporadic and familial form of the disease [108]. The age and site of onset, the neurological signs, and the duration are the heterogeneous clinical features observed in ALS patients [1]. Several cellular and physiological processes have been implicated in the development of ALS such as

cytoskeletal perturbations, oxidative stress, protein aggregation, glutamate and excitotoxicity, gene mutations, immune and growth factor dysregulation, and constitute potential therapeutic target regenerative medicine [109].

In recent years, regenerative treatments have focused on the transplantation of human neural stem cells (hNSC). Preclinical studies have demonstrated that intraspinal implantation of hNSCs provide support to damaged cells and immune modulation (for review see Ref. [106]). Phase I and II clinical trials demonstrated the safety of intraspinal human spinal cord-derived neural stem cell transplantation [110]. These studies demonstrated the potential contribution of neuronal stem cell therapy for ALS, and further studies on regenerative potential are needed. Neurotrophic factors are important factors for the treatment of neurodegenerative disorders and demonstrated some efficacy in preclinical studies for slowing the progression of the diseases. However, infusion or intrathecal delivery of BDNF failed to show any improvement in ALS patient survival in phases I/II and III clinical trials [111, 112]. The patients with insulin-like growth factor type I (IGF-1) injected subcutaneously also failed to provide benefits to ALS patients [113]. CNTF subcutaneous administration in ALS patients in a phase I clinical trial also failed to show any improvement [114]. The poor pharmacokinetic properties of these neurotrophic factors, short half-life, and low transport across the BBB limited their therapeutic efficacy.

Albeit several promising studies have used nanotechnology to encapsulate neurotrophic factors and demonstrated their potential for regenerative medicine for diseases such as AD, PD, and HD, when delivered through non-parenteral routes to bypass the BBB; no study has assessed their neuroprotective and regenerative potentials for ALS patients. Few studies have developed nanoparticles for the delivery of drugs for the treatment of ALS. Riluzole, a potent neuroprotective agent which protects neuronal cells from acute damage induced by glutamine, was encapsulated in solid lipid nanoparticles [115]. The formulation was injected into the peritoneal cavity of a rat model of ALS and demonstrated higher accumulation in the brain when compared to the free formulation [115]. The delivery of riluzole nanoparticles through a non-parenteral route remains to be assessed. Minocycline, an anti-inflammatory drug which demonstrated neuroprotective effects in vitro but showed harmful effect on patients with ALS in clinical trial due its poor bioavailability in vivo and systemic toxicity [116], was encapsulated into LPS modified liposomes and assessed in vivo in a $SOD1^{G93A}$ mouse model of ALS [117]. The minocycline-loaded LPS liposome intracerebroventricular injection delayed the onset of ALS in a $SOD1^{G93A}$ mouse model of ALS [117].

Only a few studies reported the use of nanomedicines for the treatment of ALS, and no research was reported regarding the development of the non-parenteral delivery system. The poor understanding of ALS onset and progression, the lack of biomarkers for early detection, the rapid evolution of the disease after onset, the measurement of patient improvement, the development of animal model replicating the human disease are few considerations limiting the development of therapeutic strategies.

2.5 Regeneration of impaired vision

The eye is a complex sensory organ (Fig. 2) characterized as the brain by a limited capacity for self-repair and injury or disease to the eye could be debilitating, as most blinding diseases have no treatment [118]. In recent years, nanotechnology and nanoformulations have addressed few challenges to overcome the anatomical and physiological barriers of the eye and proposed new avenues for the treatment, management, and regeneration of ocular tissue affected by degenerative diseases such as retinitis pigmentosa, age-related macular degeneration, and glaucoma.

2.5.1 Retinal diseases: Retinitis pigmentosa and age-related macular degeneration

In developed countries where preventable causes of visual impairment are routinely managed, the degeneration of the retina is the leading cause of blindness. The retina is a stratified tissue composed of more than 100 different cell types that reacts to the light, and processes the visual signals. The cone and rod photoreceptor cells convert the light into an electrochemical signal, which is carried to the brain visual's cortex through the optic nerve. Retinal disorders commonly involve the degeneration of photoreceptors; but the variety in the causes challenges the development of "a fit for all" therapy. Following is the description of two diseases associated with the degeneration of photoreceptors, and the regenerative steps undertaken to limit their progression in default of restoring the tissue to its full functionality.

Retinitis pigmentosa (RP) is a cluster of inherited retinal dystrophies associated with the degeneration of rod and cone photoreceptors, and formation of retinal pigment deposits [119]. The onset of the disease occurs anytime from infancy to late adulthood

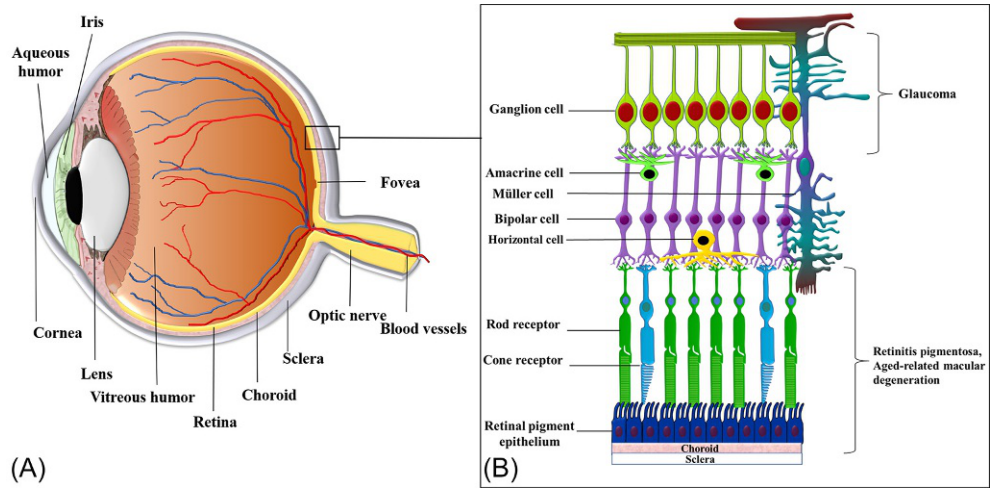

Fig. 2 Schematic representation of the human eye (A) and cellular organization of the retina affected by ocular neurodegenerative diseases (B).

[119]. RP is the most common inherited disease of the retina and affects 1 in 5000 people worldwide [120]. The development of the disease is usually bilateral, associated primarily with nyctalopia (rod degeneration) followed by the loss of peripheral vision (cone degeneration), and eventually leading to tunnel vision due to the narrowing of peripheral vision and lastly blindness [120]. More than 238 genes and 278 loci have been associated with RP [121]. The mode of inheritance for non-syndromic RP varies considerably as 15%–25% of RP cases are autosomal dominant, 5%–20% are autosomal recessive, 5%–20% are X-linked, and the remaining cases (40%–50%) are sporadic [122]. No standard treatment is prescribed for RP; vitamin A, and fish oils which were widely recommended for slowing the progression of RP failed to demonstrate clear evidence of benefit in recent studies [123].

Another type of retinal degenerative disease is the age-related macular degeneration (AMD). AMD is the most frequent cause of blindness in the elderly population. AMD affects the macular region of the retina and progresses to impair the central vision [124]. The number of people affected by the disease is expected to be around 200 million in 2020 and rise to 300 million by 2040 [125]. The disease is classified based on its progression stage as either early or late AMD; the late stages are subdivided into either geographic atrophy, neovascular (wet or exudative), or non-neovascular (dry or non-exudative) [126, 127]. The age is the main risk factor as nearly all late-stage patients are over 60 years of age and represents 5% of the population over 75 years of age [128, 129]. The risk of developing the disease is influenced by nongenetic factors such as smoking and diet as well as genetic factors as 52 genetic variants were identified spread across 34 AMD loci [130]. These genetic variants explain 27.2% of the disease variability [130]. The non-neovascular form of AMD is characterized by the accumulation of drusen while the neovascular form is characterized by the neovascularization of the retinal pigment epithelium, retina, or subretinal space from blood vessels originating in the choriocapillaris [126]. The progression of the disease to late-stage is delayed by high doses of zinc and antioxidants [131]. The treatment of late-stage neovascular AMD is based on the inhibition of the vascular endothelial growth factor (VEGF), which promotes neovascularization and increases vascular permeability. Antibodies, aptamers, or recombinant proteins were developed to target VEGF and assessed in various clinical trials (for review see Ref. [130]). The efficacy of these treatments was strongly determined by the patient vision at the beginning of the treatment as they stabilize the disease progression [130]. The treatment of geographic AMD, which accounts for one-fifth of legally blind in the United States [132], relied on antibodies targeting the complement pathway and achieved a reduction in the progression of the disease [130]. However, repeated intravitreal injection of antibodies can result in endophthalmitis or retinal detachment [130].

The intrinsic regeneration of the retina is weak or completely absent without intervention [118], In recent years, gene therapy using adeno-associated virus (AAV), cellular transplantation therapy using stem cells, and induced retinal regeneration have been developed

in preclinical studies for the repair of damaged retinas [118]. However, despite the positive outcomes for some of these preclinical studies, more research is needed to prove their feasibility for treating the human eye in clinical settings, in particular, much progress is needed to understand the differentiation process of transplanted stem cells [118]. Nanomedicine offers multiple advantages for ocular treatment such as the delivery of full genes, oligonucleotides, drugs, or peptides; the nanocarriers used are biocompatible and biodegradable; the drug-releasing rate can be controlled; inflammatory response is minimum or absent; and less frequent injections are required. Also, nanoparticles can be targeted to different cell type within the eye by varying the site of injection. Intravitreal injection targets cell in the inner retinal and ocular tissues near the vitreous while subretinal injection can target cells in the retina and retinal pigment epithelial (RPE) cells [133]. Also, nanoparticles injected in the vitreous can migrate through the retinal layers and accumulate in the RPE cells [134]. Marano et al. injected a dendrimer carrying anti-VEGF oligonucleotide (ODN-1) into the vitreous of both eyes of 8-week-old *rcs/rdy* + − pigmented rats to assess the inhibition of laser-induced choroidal neovascularization (CNV) as a model exudative AMD [135]. The data obtained by the authors suggested the efficacy of the ODN-1-dendrimer for 4–6 months compared to the 28 days of most of the current treatments [135]. Mitra et al. injected a codon-optimized genomic form of Rho (co-sgRho) encapsulated into CK30PEG10-TAT diblock copolymer nanoparticles into the subretinal space of a homozygous $Rho^{P23H/P23H}$ knock-in RP mouse model [136]. The study demonstrated the partial improvement in visual function in this mouse model of RP [136]. Cai et al. injected the wild-type retinal degeneration slow (*rds*) gene encapsulated into CK30PEG10K nanoparticles to rescue the RP phenotype of the *rds* +/− mice [137]. The phenotype of the *rds* +/− mouse model closely resembles the phenotype of patients with RDS-associated RP [137]. The retinal injections were performed on postnatal day 5, when murine retina cells are still proliferative and undergoing differentiation and maturation, or on day 22 where the retina is mature. The retinal injection of *rds* gene on day 5 or 22 was sufficient to partially rescue the RP phenotype in *rds* +/− mice, also less efficiently on day 22 without any side effects [137]. Pensado et al. encapsulated the human pre-mRNA processing factor 31 (*prpf 31*) gene into Span poly-L-arginine nanoparticles [138]. The mutation of *prpf 31* and prpf gene family are the second most common cause of autosomal dominant RP [139]. The subretinal injection of the nanoparticles containing the human *prpf 31* in heterozygous knock-in $prpf31^{A216P/+}$ mice as an animal model of autosomal dominant RP associated with PRPF31 mutations improved the visual acuity and retinal thickness [138]. Read et al. used a cell-penetrating peptide $CGGG(ARKKAAKA)_4$ conjugated to PEG to encapsulate an expression cassette for GDNF [53, 140]. In a mouse model of retinal degeneration induced by light and leading to the apoptosis of photoreceptors as observed in RP and AMD, the injection of this nanoformulation into the subretinal space was shown to reduce apoptosis and the thickness of the outer nuclear layer of the superior retina 14 days post-injury [53]. The same research

group modified the GDNF plasmid structure to extend its stability and rescued the photoreceptors in a blue light-induced photoreceptor degeneration 77 days postinjection [52] (Table 1). The same nanocarrier was used to deliver human vascular endothelial growth factor receptor 1 (*flt1*) gene [141]. The soluble form of vascular endothelial growth factor receptor 1 was shown to reduce the progression of choroidal neovascularization (CNV) in a mouse model of CNV [142]. The injection of *flt1* gene nanocarrier into the mouse retina was sufficient to reduce by 50% the CNV in a murine model of AMD [141].

The delivery of neurotrophic factors can achieve the prevention of retinal cell degeneration. Sakai et al. incorporated basic fibroblast growth factor (bFGF) into gelatin nanoparticles and assessed the protection against photoreceptor degeneration in Royal College of Surgeons (RCS) rats following intravitreal injection [41]. The intravitreal injection of bFGF was shown to rescue the photoreceptor degeneration in inherited retinal dystrophy or following light-induced photoreceptor degeneration in rats [143, 144]. A single injection of the bFGF nanoparticles prevented the degeneration of photoreceptors through the inhibition of apoptosis [41]. Audrieu-Soler et al. demonstrated that a single intravitreal injection of GDNF encapsulated into PLGA microspheres was sufficient to slow the degeneration of the retina in rd1/rd1 mice, a mouse model of autosomal recessive retinitis pigmentosa [42]. More recently, Garcia-Caballero et al. showed that a single intravitreal injection of PLGA/vitamin E microspheres loaded with GDNF and melatonin promoted the rescue of photoreceptors in rho (−/−) mice, a mouse model of retinitis pigmentosa [43]. Melatonin acts as a neuromodulator of retinal physiology and within the eye [145].

The control of the oxidative stress was shown to stabilize photoreceptor degeneration observed in disease such as RP and AMD. Chen et al. used cerium oxide nanoparticles (nanoceria) (5 nm) to scavenge reactive oxygen intermediates in a Sprague-Dawley albino rat model of photoreceptor degeneration induced by light [146]. The intravitreal injection of nanoceria before light exposure protected the photoreceptors from apoptosis [146]. The protection against photoreceptor degeneration was also shown in a *vldlr* (−/−) mouse model of neovascular AMD [147]. A single dose of nanoceria, intravitreally injected into young mice (day 28), was sufficient to reduce oxidative stress associated with neovascularization [147]. Wong et al. showed that after a single nanoceria intravitreal injection, the nanoparticles were taken up by the retina, and delayed the degeneration of photoreceptors [148]. The nanoparticles were retained up to 120 days without noticeable toxicity [148].

Scaffolds based on various nanomaterials have been developed to promote regeneration of disease tissues. The conventional approach of transplanting cells into the subretinal space has limited efficacy due to the poor integration, incomplete differentiation, and short survival of the cells. However, the transplantation of organized cell layers established on organized polymer structure may be more promising for regeneration. Either synthetic polymers such as poly-lactic acid (PLA), PLGA,

and methyl methacrylate (MMA), or natural compounds such as alginate, fibrin, chitosan, collagen, and hyaluronic acid can be used to establish a scaffold and mimic the extracellular matrix. The nanomaterials used are biocompatible, biodegradable, mechanically compatible, electrochemically stable, and with a surface chemistry allowing cell attachment and development. The scaffold structures can be made of nanofiber, nanoemulsion, nanomicelle, nanogel, or nanosheet (for review see Ref. [149]). The technology associated with the development of cellular scaffold for the regeneration of the retinal tissue is still in its early stages; for example, Klimanskaya I described the differentiation of human embryonic stem cells into retinal pigment epithelium [150]. Suzuki et al. described an electrochemical method of harvesting retinal pigment epithelial cell sheets grown on PLGA nanosheet [151]. A phase I/II clinical trial (NCT02903576) is recruiting patients to compare the subretinal transplantation of human embryonic stem cell-derived retinal pigmented epithelium (hESC RPE) cell sheet to the subretinal injection of a cell suspension of hESC RPE for patients with AMD.

2.5.2 Regeneration of the optic nerve

The optic nerve can be damaged by multiple pathologic conditions such as glaucoma and acute optic neuropathies which often occur following ischemia, trauma, inflammation, papilledema, cancers, or infections [152–154]. The insults to the optic nerve are common causes of vision loss and may originate from the disconnection of the retinal ganglion cells (RGCs) and their axons from the rest of the brain [155]. The RGCs are located in the inner layer of the retina and convey the visual signal to the brain [156]. Also, the characteristic feature of all optic neuropathies is that RGC axons cannot regenerate, leading to the functional loss of RGCs and ultimately to their death [157]. The site of injury along the optic nerve is also critical for the survival of RGCs, as the more distant the site of injury is from the eye, the more likely the environment around the site of injury will secure the continuation of the function of the optic nerve and promote less severe RGC apoptosis [158]. The current treatment of optic neuropathies such as glaucoma focuses on slowing the progression of the disease by lowering the intraocular pressure mainly by the topical administration of prostaglandin analogs [156]. Patients in whom the pharmacological approach fails, laser surgery or trabeculectomy are performed to decrease intraocular pressure by providing a drainage route for aqueous humor [156]. However, the lowering of the intraocular pressure is not sufficient to alter the progression of the disease. In recent years, new approaches were developed to promote neural tissue regeneration. Several neurotrophins, including BDNF, GDNF, NGF, and CNTF, have been identified as potential therapeutic agents for the protection and regeneration of neurons [159]. The challenge is the delivery of the neurotrophic factors to the retina for a prolonged time to achieve therapeutic efficacy. The neurotrophic factors do not cross

the blood-retinal barrier and are rapidly degraded [160]. It is now established that encapsulation into nano- or microcarriers allow controlling the delivery rate and overall pharmacokinetic. Polymers such as PLGA have been used extensively because of their biocompatibility and ability to encapsulate proteins, DNA, RNA, or chemicals. GDNF was encapsulated into PLGA microspheres and promoted Schwann cell proliferation, and neurite outgrowth in vitro while favoring RGC survival and axon growth in several animal models of glaucoma [44–47] (Table 1). GDNF-PLGA microspheres were injected into the vitreous in all these studies. Giannaccini and Usai et al. developed a magnetic nanoparticle, which consisted of a superparamagnetic central core of iron oxide surrounded by an organic coat, conjugated to NGF and BDNF [48]. The nanoparticles were injected into the vitreous of zebrafish larvae in a model of glaucoma induced by oxidative stress and RGC loss was prevented [48]. The same group demonstrated that iron oxide magnetic nanoparticles were able to self-accumulate in the retina [161]. The neuroprotection can also be improved by stimulation of the expression of endogenous neurotrophic factors. Brimonidine (BMD), an α2-adrenergic receptor agonist, is commonly used in glaucoma patients to lower IOP as an eye drop formulation [162]. Recent studies have demonstrated that BMD also stimulated the expression of several neurotrophic agents, including CNTF, bFGF, and BDNF in RGCs [163]. Nanoformulations of BMD have been developed and are currently evaluated for the treatment of glaucoma and also AMD and RP [164–166]. However, the current data support the idea that the injection of neurotrophic factors or agents stimulating their secretion offer only a short survival reprieve for RGCs [158].

Gene therapy is another strategy considered for the treatment of glaucoma. The delivery of neurotrophic factor genes using viral vectors or electroporation was shown to protect and promote regeneration of RGCs in vitro and in vivo (for review see Ref. [167]). However, the long-term efficacy of these strategies is limited for neurodegenerative diseases such as glaucoma. Neuroprotective transplantations using stem and progenitor cells modified to secrete neurotrophic factors are a more durable strategy to preserve RGCs in progressive glaucoma [159]. For more advanced cellular degeneration, replacement therapy could be considered using cellular scaffold based on synthetic or natural nanomaterials. For example, aligned silk nanofibers multifunctionalized with BDNF and/or CNTF were shown to promote rat RGCs survival in vitro [168]. Ellis-Behnke et al. reported the use of self-assembling peptides spontaneously forming nanofibers to create scaffold-like structures favorable to partial reinnervation by axons [169]. Recently, biomimetic, highly porous scaffolds mixed natural of synthetic polymers were developed to mimic the extracellular matrix closely and hold great promise for the regeneration of nerve and other tissues [170, 171]. Polybenzyl glutamate (PBG), a biocompatible peptide-based polymer fabricated as a 3D scaffold by electrospinning technique, was shown to support the growth and differentiation of RGC progenitors [171].

A lot of biological challenges still need to be conquered, but new therapeutic approaches are emerging based on the increased understanding of the intricate pathology of these degenerative diseases.

3. Conclusion

The prospect of rescuing or replacing dead or injured tissues by new ones is appealing, but its application to the CNS is even more challenging. Many degenerative diseases remain unconquerable, despite the advances in the identification of the molecular events and characterization of genes involved. The underlying causes of the diseases and intricacy of the different molecular factors remain elusive. All current therapies have failed to sustainably improve the symptoms and remarkably delay the progression of these diseases. In recent years, nanomedicine has provided new strategies to overcome some of these hurdles by targeting, protecting, prolonging, and enhancing therapies. Many of the newly developed nanomaterials defining treatment strategies have demonstrated encouraging outcomes in animal models such as gene delivery, or scaffolding for cellular transplantation. Moreover, human clinical trials will be critical to assess the validity of these approaches.

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CHAPTER 13

Electrospun nanofibers for wound healing

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1. Introduction

Skin serves as a protective impediment against the environment around the body. Due to the major loss of skin's integrity that could be vulnerable to injury or unable to heal properly, which could lead to a rise in various ailments or can be fatal. About 1.25 million people every year in the United States suffer from burns and 6.5 million due to pressure, venous stasis, or diabetes mellitus suffer from chronic skin ulcers [1]. Attention must be drawn toward the increasing population suffering from several diseases such as diabetes, obesity, venous, and arterial inadequacy that ultimately leads to chronic wounds. In fact, the condition of diabetes ulcers has already covered a peak of 10%–22% in diabetic patients [2]. The main aim of treating wounds is to prompt closure of wounds with a functional and aesthetically moderate scar. Development in cellular and molecular biology research has led to an understanding of the biological processes involved in wound repair and regeneration of tissues and has also resulted in an enhancement in wound care. However, conventional therapies are quite expensive and life-long therapies have an ulcer relapse rate of above 70% [2]. Surprisingly, there is an amazing increase in the number of researchers who are anxious to enhance the healing quality of their wounds and the investment of huge expenditure on wound care, involving various quality waxes, remarkably increasing research in areas of tissue regeneration and wound healing. Conventional therapies employed have restricted potential, which does not allow tissue re-epithelization and pivotal functions of the ruptured skin causing infection of wound and skin dehydration. Therefore, research has been carried on nanofibers which have resulted in improved skin reestablishment and have potential of enhancing cell growth and proliferation as well as capacity of delivering bioactive molecules at the ruptured site.

2. Molecular biology of wound healing

Wounds can be defined as any rupture or damage to the skin tissue due to any injury of skin or trauma or any other conditions. The wounds are classified according to the

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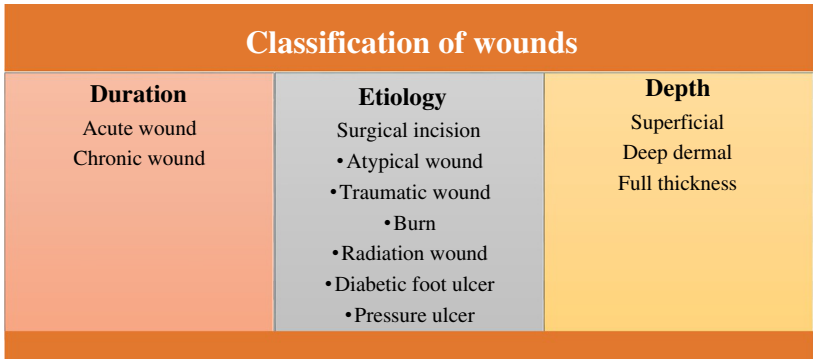

Fig. 1 Classification of wounds.

duration (acute or chronic wounds), depth of injury to the underlying tissues and skin, and etiology of the wound caused (see Fig. 1). Acute wounds generally heal in an expected timely manner and are usually a disruption of the integrity of soft tissues. The acute wound can be classified as simple or complex, on the basis of its location, size, and anatomical structures [3]. When an acute wound fails to heal in an expected timely manner it slowly turns into a chronic wound. Chronic wounds are characterized by high levels of proteolytic enzymes and cytokines, which inhibits the granulation tissue formation and epithelialization. Prevailing in this phase contributes a stable surrounding for colonization of invading bacteria and the healing phenomena are intervened by infection [3].

3. Wound healing process

Wound healing comprises three phases: inflammation, which persists up to 6 days followed by proliferation phase that continues for following 2 weeks and the third phase includes tissue re-epithelization, which is generally for 2 years. It is necessary to understand during in vivo studies there is the observance of phase overlapping because of involvement of various physiological and anatomical factors, like the foremost example of constant intercellular signaling which provides control on the release of pro-inflammatory as well as anti-inflammatory cytokines [4]. At the time of skin rupturing, it is of prime importance to quickly re-epithelize the structure and functions of the skin to maintain the body's homeostasis. Although skin itself has the power of rejuvenation, there are still several types of wounds that do not heal completely thereby giving rise to voluminous lesions or chronic wounds [5] (Fig. 2).

3.1 Inflammation

When the tissue ruptures start, there is the destruction of blood vessels, which further leads to leakage of cellular contents and extravasation of blood constituents. Hemostasis

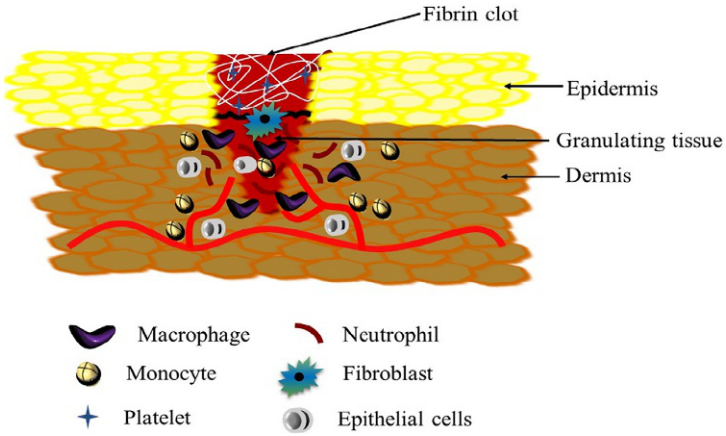

Fig. 2 Process of wound healing.

reestablishment and cell migration for the extracellular matrix is provided due to blood clot [1]. The inflammatory phase lasts for 2–5 days after skin rupturing. As soon as there is an injury to the skin, initiation of clot formation begins by intravascular platelets to cease bleeding. Moreover, these platelets are activated by thrombin and release various growth factors such as epidermal growth factor (EGF), insulin-like growth factor 1 (IGF-1), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and transforming growth factor (TGF-α and TGF-β). All these growth factors penetrate wounds and act as a biological signal, which entices neutrophils, monocytes, leukocytes, and macrophages, potentiates the inflammation, protects the wound from being infected, and secrete excessive growth factors to boost wound healing. Chemokines are proteins of small size that bind to heparin and regulates the motion of flowing inflammatory cells to the site of injury by interacting with distinct receptors [6]. Neutrophils are the most abundant type of cells, which are found in the initial phase of inflammation (48 h after injury) and diminishes after 24–36 h through apoptosis. Meanwhile, the circulating monocytes invade the wound and fully develops into tissue macrophages, thus have a very major role in the healing of wound [6].

Several chemo-attractants such as parts of extracellular matrix protein, TGF-β, and monocytes penetrate inside the wound area and change to macrophages thereby secreting various growth factors which enhance the formation of granulation tissue. Due to integrin receptors and triggering phagocytic response against microorganism presence macrophages binds to specific proteins present in the extracellular matrix [1]. The monocytes and macrophages derived growth factors are of utmost importance as they play a pivotal role in the formation of new skin tissue in wounds as a lack in the amount of macrophages in animals have compromised wound repair [1].

3.2 Proliferative phase

The proliferative phase usually involves 3 days to 2 weeks after the injury consisting of proliferation of cells. Stimulation of various proangiogenic factors such as PDGF which is secreted by platelet and several inflammatory cells inside the wound encourages the formation of new blood vessels and capillaries. Concurrently, along with angiogenesis, there are changes in the movement of fibroblasts due to the activation of PDGF and FGF from inflammatory cells in order to form granulation tissue [7]. Due to the aggregation and proliferation of fibroblasts, there is the formation of new ECM which consists of proteoglycans, collagen, and elastin. Myofibroblasts that are derived from fibroblasts also have a crucial role in the constriction of the wound area. It is also thought that triggered keratinocytes across the wounded area have a role in accomplishing re-epithelization.

3.3 Re-epithelization/tissue remodeling

Remodeling usually starts after a couple of hours of the injury. There is quick removal of epidermal cells from the appendages of skin, which, leads to the elimination of previously formed blood clots, and ruptured stroma forms the wounded area. Simultaneously, there is a pronounced phenotypic modification in the undergoing cells consisting of intracellular monofilaments, dissolution of intracellular desmosomes that provides interconnection between the cells. Thus, results from the formation of peripheral cytoplasmic actin filaments ultimately involving cell movement. After a few days of injury, the proliferation of epidermal cells begins at the wounded site behind the migrating cells. The desiccated eschar is isolated from the viable tissue through dissection of the wound by the migrating cells. This path of dissection is examined by the formation of integrin which is manifested by the migrating epidermal cells on the cell membranes [1]. With proceedings in re-epithelization, there is the reappearance of basement membrane proteins in sequential patterns from the site of the wound in the interiors of the wound with a zipper-like fashion. There is the reversal of epidermal cells to their original phenotype, which are adhered strongly to the re-established basement membrane and the underlying dermis.

3.3.1 Debridement

Debridement simply isolates the necrotic or affects tissues, which enhances the inflammatory stage and increases constriction of the wound, improves re-modeling of soft tissues, and boosting the healing process. Debridement involves surgical, autolytic, maggot, mechanical, and enzymatic methods, followed by employing wound dressings. Debridement is utilized for reducing damaged tissue and preventing the tissue from several infections. There are several disadvantages associated with debridement such as the abiding pain which is undesirable by some patients, highly qualified and knowledgeable clinicians and, specific equipment for prevention from another trauma. Hence this

procedure must be performed depending on the condition of the wound and the availability of the means required for the treatment [2].

Vascular evaluation is implemented before debridement, especially for ulcers on the lower limbs or foot whereas, debridement of limbs that are deficient of blood supply and ulcers in the proximity of bone should be avoided [8]. Surgical debridement is painful as it involves scissors, scalpel, or curette followed by local or general anesthesia. Further, enzymatic debridement agents like *Clostridial collagenase* specifically and easily degenerate the impaired tissue along with the debriding activity and they also enhance the healing of the wound. According to a study, chemical debridement of honey proved to huge benefit in Fournier's gangrene, cancrum oris, and decubitus ulcers due to the rapid healing of ulcers in these diseases and taking a longer span of time in other types of ulcers. Apart from increasing the wound healing, chemical debridement with honey also refrained the patients from general anesthesia required for surgical debridement [9].

3.3.2 Autograft and allograft

The utilization of autograft and allograft proved to be of excellent caliber in treating wounds. Autografts and allografts approach mainly harvest full-thickness fascia from a donor site of patients or other donators and graft it over the target region [2]. In a study, it was found out that split skin graft (SSG) and cultured epithelial autografts (CEA) have excellent wound healing properties and it was the only group in effectively healing the wound. The combination of SSG and CEA helps in faster healing of the wound, enhances the uptake rate of CEA, and makes an arrangement of the dermal matrix for the effective coverage of wound [10]. Autografts are known mainly for their exceptional adherence to the affected site and satisfactory aesthetic outcome, ostentatiously reducing pain, and injection simultaneously.

Moreover, there is a continuous requirement of grafts from the donor side which restricts the utilization of skin grafts and those grafts can result in undesirable scars and consequential skin contractions in the later phase of the wound healing, and also adding up the number of days in the hospital [2]. Micro-skin autografts are also widely used in the treatment of wounds caused by extensive burns. It was concluded that the Meek micrografting method is suitable in the treatment of burns in over 40% of the total body surface area (TBSA). Its application saved the lives of patients where other operative procedures do not have positive results. This method is applicable for several reasons when the other methods cannot be utilized (tissue cultures) [11]. It was also determined in a study that epidermal lipid and antimicrobial peptide results are diminished in donor skin as well as burn margin from human burn patients [12].

Negative pressure wound therapy (NPWT) is another methodology for treating chronic and infectious wounds. NPWT was found to have advanced qualities as compared to conventional wraps and compression as it increases the time period of first postoperative dressing change, reduces aggregated discharge from the grafted wound,

prolongs skin graft survival rate, lessens wound healing time, and decreases pain at the time of the first removal of the inner dressing. A study was carried out using NPWT which concluded that the aggregation of exudation was remarkably reduced by using NPWT than that of utilizing traditional gauze prior to postoperative dressing modified after micro-skin autografts [13].

The potential advantage of an allograft is that it provides wound from dehydration although which is temporary and prevents the wound from any external factors to avoid contamination of the wound. Human amniotic membranes allografts have enhanced the healing rate of burns, chronic wounds, and ophthalmic injuries. Amniotic allografts contain amnion and chorion, which consists of variable cytokines. These cytokines are to playing an important role in wound healing. Moreover, it was concluded that processed tissues retain a high content of growth factors within the membrane allografts. And chorion membranes possess a four- to fivefold higher growth factor than amnion per equivalent surface area, largely due to chorion's greater thickness [12, 14]. Dehydrated human amnion/chorion membrane (dHACM) laminates are effective treatments to promote healing of refractive wounds, which are used for the retention of biologically active growth factors during the processing of amniotic tissues. Amniotic tissues are critically important in preserving the bioactivity of the native tissues for wound care [14]. It was observed that amniotic membrane shows promise in treating chronic wounds unresponsive to traditional therapies within a formal wound clinic environment directed by evidence-based treatment algorithm guidelines. Amniotic membrane allograft is not indicated for the routine treatment of wounds. Similar to other advanced biologic treatments, it is indicated for those wounds that demonstrate a pattern of resolution that indicates where a nonhealing wound environment is present [15].

4. Recent advances in wound therapy

Currently, there are many strategies are available for managing and treating wounds such as debridement, autografts, and the application of therapeutic vehicles. Additionally, there are new therapies such as stem cell therapy, gene therapy, photothermal, and photodynamic therapy that plays a pivotal role in a few of the complicated wound treatment. Wound dressings have been conventionally used as a protective tool in the wounded area thereby isolating it from external pollution. Advances have been achieved in recent years and hence classic wound dressings (e.g., cotton and wool) are replaced by more enhanced dressings that have the potential of maintaining a secured environment around the wounded site as well as delivering some important bioactive molecules helpful in treating wounds. The aim of wound management is to prevent wounds from getting infected, speed up of wound healing, and decrease scars and pain for patients. The ideal wound dressing must be biocompatible, semipermeable to water and oxygen, nonallergenic,

and cost-effective. Also, it should enhance tissue renewal and should not cause trauma or another injury upon removal of the dressings.

4.1 Topical drug delivery

Topically applied formulations have potential advantages of wound filling by initiating genesis of blood vessels and capillaries and preventing the wounds from being infected. Hence, topical delivery systems play a key role in the healing of the various types of wound. The researchers across the globe have been focusing on developing the novel therapeutic agents for managing and treating wounds topically. Topical formulations contain several growth factors as well as antimicrobial agents due to their effectiveness in the re-epithelization of the skin structure and functions. Moreover, topical delivery of antimicrobial agents in the form of controlled release is also found to be advantageous in managing chronic and extensively colonized wounds.

Growth factors are polypeptides and monitoring of cell differentiation, migration, and play an important role in each phase of wound healing. These growth factors have an astounding effect on the enhancement of wound healing and revamping skin functions without causing any adverse effects. Some of the currently available topical formulations for treating several wounds (Table 1).

4.2 Nanotechnology for wound healing

Nanotechnology has tremendous capacity in increasing the biological efficacy as they have the potential of preventing the drug from degradation and extended the drug release. Nano-based drug delivery systems are growing areas of research in wound healing and utilized in enhancement of wound healing and re-modeling of skin, cost-effective prepared microspheres, liposomes, nanoparticles, nano-hydrogels, and nanofibers [25–28].

4.2.1 Microspheres

The bio-adhesion and swelling properties of small-sized microsphere capability have a wide range of applications in several formulations in wound healing. Due to the above property of microspheres, it can be combined in controlled-release formulations and can be prescribed for long-term treatment of wounds. Spray drying techniques are amendable to preparation such as to produce microparticles with a rough surface. This approach is considered a promising approach for burn wounds healing because they adhere better to burned skin, thereby increasing the contact surface, allowing a better release of the formulation components in the injured area [29–32]. The chronic wounds have complex physiology and there is a need for multiple drugs for ensuring proper healing of the wounds to prevent them from further infection. Therefore, multiple drugs can also be given formulating microspheres and entrapping the drug within the microspheres.

Table 1 Marketed formulations for treating wounds.

Formulation	Therapeutic moiety	Active against	Indication	References
Neosporin ointment	Neomycin, bacitracin, polymyxin	Gram-positive and Gram-negative bacteria	Minor cuts and abrasion, prevent infection and promotes wound healing	[16]
Silverex heal gel	Silver sulfadiazine	Broad spectrum antibiotic	Minor burns, sunburn, blisters, abrasions, scratches	[17]
Soframycin	Framycetin sulfate	Gram-positive and Gram-negative bacteria	Minor cuts, lacerations, abrasions, wound dressings	[18]
Megaheal gel	Nanosilver colloid	Wide spectrum antibiotics	Minor cuts, scrapes, burns, treat skin irritation	[19]
Sudocrem antiseptic cream	Zinc oxide, benzyl alcohol, benzyl benzoate, benzyl cinnamate, lanolin	Antifungal and antibacterial	Sore skin, treating nappy rash, eczema, acne, minor burns	[20]
All terrain antibiotic gel	Bacitracin zinc	Broad spectrum antibiotics	Skin protectant, minor cuts, burns, scrapes, chafed, and chapped skin	[21]
Hydroheal gel	Carbomer, propylene glycol, silver colloid, triethanolamine	Wide range of bacteria, fungi, virus, parasites	Wound hydration	[22]
Eco-dent oral wound mouth cleanser	Sodium bicarbonate	Antifungal, prevents acidophilic bacterial growth	Minor wounds in mouth, minor gum inflammation	
Cipladine powder/ ointment	Povidone iodine	Broad spectrum antiseptic	Vaginal infection, minor cuts, grazes, abrasions, blister, burns	[23]
Cleargel Ap gel	Adapalene, Clindamycin	Gram-positive bacteria	Burns, skin irritation, and redness, blisters	[24]

Asiaticoside-based microspheres is a novel preparation with excellent regenerative healing and anti-scar effects for wound therapy by accelerating re-epithelization, regulating the synthesis and disposition of different types of collagens as well as stimulating the angiogenesis [33]. In vivo evaluation in a splinted mouse with full-thickness excision model in which PLGA-curcumin nanoparticles treated group showed nearly complete recovery by the 10th day whereas, curcumin only and PLGA nanoparticles only treated groups showed only 75% recovery during the time [29].

Due to the migration of the microspheres from the site they create inadequate support to the tissues. Shamloo et al., performed a study and observed that sustained release of basic endothelial growth factor from poly(ε-caprolactone) (PCL) microspheres embedded in the hydrogel structure effectively accelerated the wound regeneration time, especially in early stages of wound healing. In the hybrid hydrogel/microsphere drug delivery system, a wound closure of 50% was achieved after 4 days. The histological analysis represented the sustained release of bFGF from PCL microspheres facilitating wound healing and angiogenesis [34]. Microspheres-embedded scaffolds showed antimicrobial activity against both Gram-positive and Gram-negative bacteria by the activity of either chitosan or sericin. These formulations also promoted wound healing probably by the sustained release of sericin [35]. The microspheres-embedded scaffolds displayed reticulated pore design whereas the microspheres were dispersed uniformly in the exterior part of the scaffold. Magnetic nanofibrous microspheres were also developed using chitin nanofibers which displayed antibacterial activity and enhanced wound healing rate. Silver conjugated ferric oxide nanoparticles exhibited remarkable and long-term antibacterial activity against Gram-negative and Gram-positive bacteria [36]. Although microspheres have extensive applications in wound healing it still has few demerits that are rapid migration of the microspheres from the site of application, variable release rates, and embolism which limits their utilization.

4.2.2 Liposomes

Liposomes are bilayer vesicles comprising of phospholipids and cholesterol and have a tendency of incorporating both hydrophilic as well as hydrophobic drugs, therefore liposomes have been given wide attention as a nanocarrier for drug delivery. As liposomes entrap the hydrophilic moiety in the innermost aqueous cavity and the hydrophobic moiety in the lipid bilayer, they can prevent the leakage of the drug thus can be employed as a sustained-release system. Moreover, as liposomes cover the wounded site effectively, they prevent the wound from getting dehydrated by keeping the wound area moist thus promoting wound healing. Therefore, keeping into consideration each benefit of liposomes they have been excessively utilized in skin regeneration and enhancing wound healing. The drug release behavior of the liposomes exhibited a biphasic pattern, with the burst release at the initial stage and sustained release subsequently [37–40]. Thus, with the help of a study on the sustained release of quercetin-loaded liposomes, it is concluded

that they display justifiable stability. The role of glucocorticoid as an anti-inflammatory was assessed by formulating dexamethasone phosphate-loaded liposomes and the effect was observed on the chronic wounds and the rate of healing of the wounds [41]. Madecassoside obtained from *Centella asiatica* has an excellent role in treating various skin disorders such as wounds and psoriasis but has poor membrane permeability. Therefore, the development of madecassoside liposomal formulation enhancing the permeation rate and thus achieving excellent wound healing capacity. In vitro percutaneous absorption and diffusion, experiments showed that the liposomes prepared by the double-emulsion method possessed the maximum transmittance and the largest capacity of storage of MA in the skin, which made MA to be absorbed and permeated quickly by the skin [42].

Curcumin-loaded liposomes might be proposed as a promising preparation in enhancing the healing effect of various wounds [43]. Novel liposomes with SF hydrogel core was successfully developed by the common liposomal template, followed by gelation of liquid SF inside vesicle under in situ sonication [44]. It was observed that liposome along with SF hydrogen core can efficiently incorporate basic endothelial growth factor in the innermost hydrogel core and can avoid rapid leaking inside the wound simulated fluid. As per literature, it was observed that the liposomes not only synergistically enhanced the cell proliferation and migration, but noticeably boosted wound healing and improved the healing quality when both incorporated into an ointment matrix [2]. Therefore, the combination of liposomal ointment has ability to treat the wounds caused by burns. Chitosan-based liposomal formulation was also developed to determine the healing efficiency of the wound and has found to be of great advantage in diabetic wounds. With the help of the study, it was concluded that the chitosan coating improved the stability of liposomes in physiological conditions and modified the release of SP in a "programmable" way [45]. Nevertheless, liposomal preparations still have several drawbacks such as raid leakage of drugs, less reproducibility, and their stability restricting their evolution in clinical use.

4.2.3 Nanoparticles

Nanoparticles (NPs) have been developed in recent years due to their rapid healing of the wound, sustaining the drug release, protecting the drug from getting degraded by various enzymes, gaining popularity in the areas of bioengineering and biomedicine. NPs are formulated such as polymeric NPs, lipid NPs, gold NPs, and silver NPs till date and continue to grow for treating infectious and chronic wounds. These nanoparticles are biocompatible and are proving effective for the delivery of various antimicrobial drugs at the wounded site and enhancing the rate of wound healing. Hence extensive research is carried out for entrapping antimicrobial drugs in the nanoparticles. The broad range of polymers and metals are employed in the formulation of different nanoparticles such as chitosan, PLGA, gold, silver, etc. and promoting the rate of wound healing. Lipid nanoparticles such as solid lipid nanoparticles and nanostructured lipid carriers utilize pure

lipid molecules and their development does not involve the use of organic solvents, hence there are no toxicity issues of such prepared lipid nanoparticles [38, 46–49].

Tian et al., investigated the antimicrobial effect of silver nanoparticles on the wounds and the role of cytokines involved in the inflammation. They not only confirmed the efficient antimicrobial property of silver nanoparticles but also implicate the ability of silver to modulate the cytokines involved in wound healing [50]. Several growth factors such as vascular endothelial growth factor and the basic endothelial growth factor have an activity in the healing of diabetic ulcers. Therefore, a study was performed by developing nanoparticles loaded with growth factors. VEGF and basic endothelial growth factor-loaded nanoparticles were incorporated into the fibrin matrix of the scaffold to protect the PLGA nanoparticles from leakage and to avoid the burst release in the initial phase [51]. Hence, this therapy delivers the growth factors to the specific wounded site and maintains their residence at the site thus preventing the frequent application of the formulation. Due to the wide activity of growth factors in wound healing, extensive studies are performed on nanoparticles loaded with several growth factors. Xie et al., investigated the role of growth factors by encapsulating polymeric nanoparticles in electrospun fibers and determining the release pattern. Xie and coworkers concluded that VEGF helped angiogenesis in an early stage of the healing process, while PDGF-BB improved the epithelium regeneration, collagen deposition, and functional tissue re-modeling [52, 53]. Thus, growth factors have an immense ability in the treatment of chronic wounds especially diabetic wounds and can be of great use in the near future.

Chereddy et al., demonstrated that the PLGA-based sustained delivery of LL37 significantly improved the wound healing activity compared to PLGA or LL37 alone [54]. Due to the strong antibacterial property of silver, it is widely used in various formulations for healing infectious wounds and the management of chronic burn wounds. It was observed that after the application of Ag NP-based dressing for an extended period (17 days), the organized skin structure (dermis and epidermis) was re-established in a previously unhealed part of the wound [55]. Antioxidants also have a role in the cutaneous wound healing, therefore the role of epigallocatechin gallate (EGCG) and α-lipoic acid (ALA) as an antioxidant was investigated on the healing rate of wound and skin re-epithelization. Leu et al. suggested that AuEA significantly accelerated mouse cutaneous wound healing through anti-inflammatory and anti-oxidative effects [56]. The receptor for the advanced glycation end product (RAGE) emerges enormously in the case of diabetic wounds. Thereafter, a study was performed for confirming the linkage between appearances of RAGE with antioxidants encapsulated in a gold nanoparticle. The results suggest that the combination of AuNP, EGCG, and ALA significantly accelerated diabetic cutaneous wound healing through angiogenesis regulation and anti-inflammatory effects [57].

Opioids are well known for providing relief from extreme pain. Hence, various opioids are encapsulated inside lipid nanoparticle and their effect was observed. Opioids are

thought to induce the movement of keratinocytes from surface to the center of the wounds which have a role in wound healing and tissue re-epithelization. With the help of study, it was concluded that acceleration of wound closure, low cytotoxicity, and irritation as well as possible prolonged morphine release make SLN an interesting approach for innovative wound management [58]. Nanostructured lipid carriers of andrographolides were developed and encapsulated inside the chitosan-hyaluronic acid scaffold which resulted in improvement in wound healing rate leaving behind no scar and increased tissue quality when evaluated in rats [59]. Although nanoparticles have great advantages in treating several types of wounds and preventing them from getting infected, re-establishing and improving the quality of soft tissues with minimal or less sign of scar but still they suffer from disadvantages such as their toxicity, discoloration of the skin in case of applying silver nanoparticles.

4.2.4 Nanohydrogels

Nanohydrogel is the three-dimensional polymeric networks considered as an ideal formulation for wound management: the porous three-dimensional structure endows it with the ability of aqueous fluid absorption, preventing wound dehydration, and creating a beneficial moist environment for wound healing; its nonadhesive nature allows it to preserve the wound bed while maintaining the penetration of oxygen, which is necessary for wound healing; the soft texture of nanohydrogel provides comfortable experience in the course of treatment [2]. Hence, nanohydrogel has got attention in current years due to their ability to absorb tissue exudates and their porous nature helps to entrap drugs and deliver them in a controlled manner.

Anjum et al. developed a composite material for wound dressing consisting of nanosilver nanohydrogel (nSH) by coadministration of *Aloe vera* and curcumin. Further, they studied their individual effects as antimicrobial, promotion of wound healing, and prevention of infection. The results indicated that *Aloe vera* dressing was the most effective one and could be a promising candidate for wound dressing [7]. A gellan-cholesterol nanohydrogel embedding baicalin was introduced to speed up the wound healing process [60]. This nanohydrogel was characterized by its viscosity which enhanced retention of the formulation in the skin and hence was further utilized to cover cutaneous wounds in mice. Zhu et al. reported the rapid hemostasis capacity and increased wound healing capacity by developing hydrogel of chlorhexidine diacetate-loaded nanogel [61]. One more study was conducted to determine the potential of a hydrogel on second-degree burns. It was concluded that the developed hydrogel composite displayed no toxicity and enhanced healing rate of wounds by reducing the levels of TNF-α and enhancing the amount of MMP-2 within the wounds [62]. Besides nanohydrogels have a wide range of applications in the cutaneous wound therapy and burns, they are of high cost and are nonadherent, and they require support by a secondary dressing, get rapidly degraded, and have compromised mechanical properties.

4.2.5 Nanofibers

Nanofibers are made from natural as well as a synthetic continuous chain of polymers representing the shape of nanofibrous sheets mostly utilized in tissue engineering. These nanofibers are so constructed that it imitates extracellular matrix and maintains suitable surrounding for cell attachment and enhances the interaction between cells and the drug, providing a substitution for dermal analogs. Nanofibers have vast applications in the field of tissue engineering, wound healing, prevention of adhesion, cancer treatment, and are proving the most efficient delivery system for the treatment of the same. There are various techniques available for the manufacturing of nanofibers which include bicomponent extrusion, phase separation, template synthesis, drawing, melt-blowing, and electrospinning.

Bicomponent extrusion

Releasing two polymers together in the same fiber from the same spinneret is defined as bicomponent extrusion. Basically, these fibers are spun from the mixture of two polymers in the defined ratio. Rapid cooling of the fiber below the holes of the spinneret is a major attribute in the fabrication of fibers. The differences in spinnability between the two polymers would almost hinder the spinnability of its blend, with the exception of lower mixtures concentration ($\leq$20%) [63]. In the bicomponent extrusion method, two polymers are delivered to the hole of the spinneret that is divided by blade edge or a septum, feeding the two segments into side-by-side arrangements. The high-speed bicomponent spinning of poly-(ethylene terephthalate) (PET) (core) and poly(propylene) PP (sheath) was carried out and the structure of the as-spun fibers was investigated. A detailed description of core/shell bicomponent nanofibers and the advances in the field is explained by Naeimirad et al., which consists of the manufacturing technology and the applications of the bicomponent nanofibers [64].

Phase separation

Here, in phase separation, a polymer is initially mixed with a solvent before subjecting to gelation. The major mechanism in this system is the separation of phases owing to physical inconsistency, therefore extracting the solvent phase leaving behind the other residual phase [63]. The method for the manufacturing of poly(L-lactic) acid (PLLA) nanofiber has been described in five chief steps, viz., polymer dissolution, gelation, solvent extraction, freezing, and freeze drying [65].

Template synthesis

Template synthesis is the most commonly used method for the manufacturing of nanofibers and carbon nanotubes. A template is required for the synthesis of a desired component or structure. Therefore, DNA replication and casting techniques are included under template synthesis. In template synthesis, there is a use of water pressure and

permeable membrane for managing the extrusion of the polymer, which when gets associated with the solidified solution renders nanofibers of the size governed by the permeable membrane. Carbonaceous nanofibers as a monolithic hydrogel have been developed by using a template synthesis method involving hydrothermal carbonization process which has found to be of having applications in a broad range of fields [66].

Drawing

The drawing method is only utilized for the materials which are viscoelastic and have a higher degree of deformation, yet these materials must have some strength to maintain the stress throughout the pulling. At the molecular level, the drawing method can basically be categorized as dry spinning. An ordinary process involves the surface of silicon dioxide, a micromanipulator, and micropipette for producing nanofibers. Although this method is used to produce nanofibers on a laboratory scale individually thereby hampering the production on the industrial scale. This micropipette with the help of a micromanipulator was dipped into the droplet close to the contact line. The nanofiber was made by withdrawing the micropipette from the solution around the speed of 1×10^{-4} m/s [63]. This nanofiber drawing was commonly repeated on every droplet. The viscosity of the material on the surface of the droplet increases with evaporation. Therefore, while drawing a nanofiber, consisting of a viscoelastic material which sustains the strong deformation it becomes necessary to hold the stress that is developed throughout the pulling of the nanofiber.

Melt-blowing method

The melt-blowing method is composed of the production of fiber involving single step by a solution of polymer expelling through an orifice die and moving down the expelled with the hot air, generally at a homogenous temperature as like that of the stream of the molten polymer. The air helps in attenuating the expelled melt into fibers by exerting force, thus are assembled in the form of an unwoven mat. The thermoplastic polymer in a sufficiently economic spinning method can be employed in the melt-blown technology.

Electrospinning

Electrospinning is the most common method used for the manufacturing of polymeric nanofibers. This method is simple to apply as well as a smart method to produce unwoven mats giving an excellent volume/area ratio. Here, a stream of the polymer melt is expelled from the tip of the droplet which is under electrostatic forces. The length of the fragments of the single fiber is of several centimeters that can be assembled and gathered (Fig. 3).

Electrospun nanofibers have gathered wide and extensive popularity in every field such as biomedicals, environmental, implants as well as in the rapid closure and healing

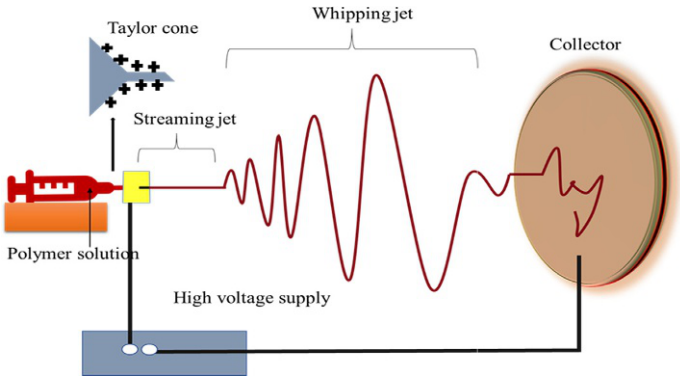

Fig. 3 Electrospinning process.

of wounds. Electrospun nanofibers scaffold offers advantages such as higher surface to volume area ratio, reticulated nanoporosity, sustained release of the molecules, adequate cellular respiration, high flexibility, easy assimilation of proteins, and hence all these properties make them as an ideal nanofiber for utilization in the healing of the wound. The most important property of any scaffold for the treatment of wound is that it should imitate the extracellular matrix which is given by the electrospun nanofibers scaffold making it of prime consideration in tissue engineering meant to be used in tissue engineering because it has an impact on cell binding [67]. A study was carried on collagen nanofiber made by the electrospinning process which was assessed for wound healing activity. The collagenous nanofibers were then evaluated by the size of the distribution of the pores, excellent mechanical strength, higher surface area to volume ratio which proved favorable factors for the attachment of cells, cellular growth, and their proliferation [68]. Some of the electrospun nanofibers incorporated with therapeutic agents by using polymers are listed in Table 2.

The nanofibers of poly(ε-caprolactone)-poly(ethylene glycol) and poly(ε-caprolactone) were electrospun and developed for surface conjugated chemically for rhEGF and was evaluated by XPX spectroscopy and were concluded that it heals the wound rapidly and effectively [95]. Use of various synthetic and natural polymers for the development of effective nanofibers is been carried out to improve the quality and rate of wound healing along with no signs of scars or any marks left behind on the skin and to accelerate rapid tissue re-modeling. The number of polymers utilized in the development of electrospun nanofibers for efficient healing of wound and skin regeneration is as discussed below.

Natural polymers Scaffolds of electrospun nanofibers developed by utilizing natural polymers display magnificent properties such as biocompatibility, biodegradability as well as many other such biological properties which are characterized by cells for their

Table 2 Summarized tabular form of drugs which are formulated into nanofibers.

Therapeutic moiety	Polymer used	Outcome	Reference
Quaternary ammonium salts	Polyacrylonitrile	Good antibacterial activity	[69]
Triclosan	Cyclodextrin	Better antibacterial activity against both bacteria	[70]
Silver nanoparticles	Chitosan, polyvinyl alcohol (PVA)	Superior properties and synergistic antibacterial effects	[71]
Silver nanoparticles	Chitosan, Poly(ethylene oxide) (PEO)	Enhanced the inactivation of bacteria	[72]
Triclosan	Polylactide	Longer lasting antimicrobial activity	[73]
Silver nanoparticles	Carboxymethyl Chitosan	Excellent antimicrobial activity	[74]
Silver nanoparticles	Poly(vinyl alcohol), Chitosan	Higher antibacterial activity was observed in the nonwoven mats	[75]
Plantaricin 423	Poly(D, L-lactide), Poly(ethylene oxide)	Controlled antimicrobial delivery systems	[76]
ZnO/TiO_2	Poly(methyl methacrylic acid)	Enhance antimicrobial activity	[77]
N, *N*, *N*-trimethylchitosan iodide	Poly (caprolactone), Poly(acrylic acid)	Effective against both Gram-positive and Gram-negative bacteria	[78]
N-(2-hydroxyl) propyl-3-trimethyl ammonium chitosan chloride and N-benzyl-*N*, *N*-dimethyl chitosan iodide	Chitosan, poly(ethylene oxide)	Better inhibition of antibacterial activity	[79]
Triclosan	Poly(lactic acid)	Efficient antibacterial property	[80]
Doxycycline hyclate	Poly(acrylic acid)	More efficient properties	[81]
Mefoxin, Cefoxitin sodium	Poly(lactide-*co*-glycolide)	Prolonged release of drug	[82]
Chitosan	Polyacrylonitrile	Enhanced antibacterial properties	[83]
Polyhexamethylene biguanide	Cellulose acetate and polyester urethane	Long-term antimicrobial effect	[84]

Table 2 Summarized tabular form of drugs which are formulated into nanofibers—cont'd

Therapeutic moiety	Polymer used	Outcome	Reference
Silver nanoparticles	Nylon	Good photocatalytic and antimicrobial properties	[85]
7,7,9,9-tetramethyl-1,3,8-triazaspiro [4.5]-decane-2,4-dione	Polyacrylonitrile	Potential disinfectant	[86]
Honey, Chitosan	Polyvinyl alcohol	Effective wound dressing and no toxicity	[87]
Ampicillin	Poly(methyl methacrylate), nylon	Sustained drug release	[88]
Potassium 5-nitro-8-quinolinolate	Poly(ethylene oxide), chitosan	Good antibacterial and antimycotic activity	[89]
Lidocaine, Mupirocin	Poly-l-lactic acid	Sustained release	[90]
Ciprofloxacin hydrochloride, Levofloxacin hemihydrate, Moxifloxacin hydrochloride	Poly(L-lactide-*co*-D, L-lactide), Poly(ethylene glycol)	Suitable for slower release	[91]
ZnO	Nylon6	Excellent antibacterial efficiency	[92]
Amoxicillin	Laponite-doped poly(lactic co-glycolic acid)	Good cytocompatibility with the sustained release	[93]
Silver Nanoparticles	Poly(vinylidene fluoride)	Good antibacterial activity	
Sericin	Chitosan	Good bactericidal activity	[94]

biological process. Several natural polymers such as hyaluronic acid, collagen, gelatin, silk fibroin, and chitosan have displayed enormous application in wound healing and skin re-epithelization as a nanofiber scaffold. Collagen is the main component of the extracellular matrix is found largely in animals and is an insoluble fibrous protein that maintains and gives the tensile strength as well as structural rigidity and integrity to tissues. In a recent study, it was proven that nanofibers produced from natural polymers are well tolerated and are effective than gauze and collagen sponges in the healing of the wound. The nanofibrous membrane was developed by using collagen as well as chitosan which

increased the rapid healing of the wound and influence the cellular movement as well as proliferation [96–99]. Nanofibers of PLGA/collagen were also developed and investigated on the rate of wound healing which resulted in advanced healing of the wound at the early phases as the nanofibers increased the pace of healing of the wound [48, 100]. Nanofiber of poly(ε-caprolactone) was synthesized and laminated with collagen which displayed the effects on dermal fibroblasts as well as maintained stability while handling and displayed structural as well as mechanical integrity and provided a base for adhesions of the cells [101].

According to a study, electrospinning factors like a syringe, speed of the pump, the concentration of the solution, the voltage applied, the rotation speed of the mandrel, and several other factors must be kept into consideration before the development of the nanofibrous membrane [102]. There is a need for optimization of every method for producing the nanofibers especially collagen type II to attain the required diameter of the fiber, breadth, orientation, and porosity of the scaffold. With the help of co-electrospinning nanofibers of collagen and zein were developed in a solution of acetic acid which resulted in improve electro-spinnability of collagen as well as enhanced the cell adhesive ability by modifying the ration of collagen is to zein [103]. Nanofibers of collagen and chitosan complex were produced which could be favorable for impairing ruptured tissue and to imitate the biological extracellular matrix thus producing the nanofibers having sufficient stability and appropriate diameter [104].

Gelatin is another polymer derived from collagen, used in the production of electrospun nanofibers which is acquired by partial hydrolysis and is less immunogenic than collagen. The use of gelatin in wound healing is limited due to the complex mechanical properties and quick degradation of the gelatin [5, 67]. Gelatin grafted poly (ε-caprolactone) nanofiber was evaluated which displayed a strong potential and governed the alignment of endothelial cells directing toward the fibers [105]. Researchers confirmed the concentration of the gelatin and highly influence the properties of the nanofiber scaffolds and hence varying concentrations of poly(ε-caprolactone) and gelatin was prepared to study the effect of the various ratios on mechanical and biodegradable properties [106]. Gelatin nanofibers were also made by using fluorinated alcohol, i.e., 2,2,2-trifluoroethanol in which gelatin was dissolved which is supposed to increase and allow new opportunities as a flexible polymer in the fields of tissue engineering scaffolds, drug delivery carriers, and hemostatic fibers [107]. Cross-linking of gelatin is performed for improving the mechanical properties and enhancing the hydrophobicity of the resulting nanofibrous membrane. Concisely, a mixture of gelatin with several polymers enhances the affinity and efficacy of the nanofibrous scaffolds by improving the biodegradability and biocompatibility of the fiber [108]. Gelatin-based wound dressings were developed incorporating epidermal growth factor and were assessed for their healing properties on the rabbit skin [109]. In situ hydrogels using alginate and gelatin were formulated by eliminating the use of crosslinkers which provided a moist environment at the wounded site and resulted in enhanced wound healing and tissue re-modeling [110].

Hyaluronic acid is one of the naturally occurring polysaccharides and the main constituent of the extracellular matrix. Hyaluronic acid has proved to be advantageous in healing wounds and plays a crucial role in maintaining keratinocyte movement and proliferation thereby modifying the inflammatory response and decreasing the formation of any scars. Hyaluronic acid scaffold was developed which was found to be safe and highly efficient in wound closure. Various growth factors such as fibroblast growth factors were incorporated in the scaffold for enhancing tissue repairing and rapid closure of wounds [111]. The addition of fibronectin and hyaluronic acid at lower concentrations in collagen-based sponges influences the number of fibroblasts in the proliferative stage which accelerates the rate of closure of wounds and improves skin re-epithelization [112]. Hyaluronic acid has been extensively used in wound healing and tissue re-modeling. It has also shown to reduce the amount of collagen deposition at the wounded site while the formation of the scar and beneficial in revamping the structure of the extracellular matrix [5, 113]. Moreover, the degradation product obtained from fibrin and hyaluronic acid has also been found to be of use in controlling the cellular level functions that play a role in the inflammatory response and help in angiogenesis in the closure of wound [114].

Similarly, chitosan is also used in making wound dressings and thought to have a role in treating burns due to their hemostatic, antibacterial, rapid wound closure, and antifungal parameters. Chitosan is a polysaccharide obtained from the deacetylation of chitin and is found abundantly in nature. It was also confirmed that chitosan is thought to play a role in inflammatory responses thereby regulating tissue granulation and organization process and hence widely used in treating open and large wounds in animals, in spite of various complications on the dose of the chitosan to be used in the animals [115]. Chitosan has also been useful in tumor activity by stimulating macrophages and provides a surface for the growth of the tissue. Also, the hemostatic nature of chitosan is beneficial in decreasing pain and enhancing the clotting of blood. Chitosan is also thought to decrease the formation of scars and rapid closure of wounds by the accumulation of collagen and hyaluronic acid at the wound site [116]. Chitin as well as chitosan can be easily formulated as micro/nanoparticles, hydrogels, beads, scaffolds, nanofibers for the applications in drug delivery, gene therapy, tissue engineering, and wound healing [117]. The use of chitosan and its derivatives have also been used against many bacterial and fungal infections thus enhancing the applicability as a wound dressing in order to prevent the growth of infection in the wounded area thereby preventing further infection and increasing the rate and quality of wound healing [116, 118]. In a study, it was also concluded that the oligomers of chitosan potentiate better-wound healing than chitosan. The wound breaking strength and the action of collagenase found in the chitosan group were thought to be more than the chitin group [119].

Silkworm generates the fibrous type of protein and called silk fibroin. Currently, more huge attention is given to silk fibroin for wound closure and healing due to its

distinctive properties of quick tissue revitalization, better biocompatibility, reduced immunogenicity, reduction of scars, hemostatic response, and increased collagen biosynthesis. Attempts are made to produce nanofibers scaffolds for application in wound healing. A study was performed to evaluate the effect of silk fibroin as a nanofiber scaffold and was concluded that silk fibroin and alginate in combination have an excellent outcome compared with using alone silk fibroin or alginate which is brought about by re-epithelization through the quick proliferation of the epithelial cells [120]. Nanofiber scaffolds using silk fibroin and elastin were also investigated for the healing of wounds in burns which resulted in effective dermal healing of wounds evaluated on a full-length thickness wound, compared with the standard burn wound [121]. The antimicrobial peptide of cys-KR12 of prepared and immobilized onto the nanofibrous scaffold of silk fibroin and was evaluated for multiple biological activities which resulted in enhanced in the closure of the wound and can be a promising candidate as a wound dressing material [122]. Using silk fibroin, nanofibers displayed an enhanced efficacy in treating burn wounds by reducing inflammation, increasing the stimulation of growth factors at the wounded area, and enhancing the tissue re-epithelization thereby enhancing the rapid closure of wounds [123].

Synthetic polymers Synthetic polymers have been widely used in the formulation of electrospun nanofibers as they are more advantageous over naturally occurring polymers. Synthetic polymers can be modified accordingly and thus can have a broad range of applicability in any field. Synthetic polymers can improve mechanical and degradation properties by modifying the chemical structure, polymerization with other polymers, crystallinity, molecular weight, etc. The most common biodegradable polymer is aliphatic polyesters that have extensive use in biomedicals due to their mechanical strength, harmless degradation products, and easy processability. There are numerous polymers approved by the US food and drug administration (USFDA), which have been extensively used in the tissue engineering and wound healing including poly(lactic acid) (PLA), poly(ε-caprolactone) (PCL), poly(lactic-*co*-glycolic acid) PLGA, poly(ethylene oxide) (PEO), polyurethane (PU), etc. [124–128]. Synthetic polymers are usually modified with bioactive agents and then utilized in the wound healing application.

PLGA is widely used as a biocompatible synthetic polymer in various wound dressings and excellent results have been obtained by utilizing PLGA polymers. PLGA nanofibers were prepared and tripeptides were incorporated arginine-glycine-aspartic acid and were investigated for osteocompatibility of tibial wounds in rats [129]. Similarly, the blend of PLGA and collagen nanofibers was produced by electrospinning method for the initial stages of the closure of the wound and was found to be very useful [100]. Electrospun nanofibers of PLGA and chitosan/PVA were made simultaneously but were electrospun from different syringes finally making a nanocomposite fiber of the long diameter of PLGA and a defect in beads found in chitosan/PVA nanofiber and hence it was thought

that after combining the composites of PLGA-chitosan/PVA nanofiber, it would result in excellent wound closure efficacy and enhance tissue re-epithelization [130].

PCL is a biodegradable and biocompatible polymer which has gained a wide range of huge interest as a wound dressing component and is approved by the USFDA by numerous in vitro and in vivo studies to determine the safety and efficacy to be utilized in medical and drug delivery. Currently, PCL is considered to be soft as well as hard material which is found to be biocompatible with the tissues. Blend of PCL/gelatin polymer was used in producing electrospun nanofiber that resulted in enhancement of growth factors as well as cell proliferation resulting in skin regeneration thereby increasing the wound healing rate [131]. The mixture of PCL-PEG polymer was developed in such a way that the amine groups were present on the surface of the developed nanofibrous composite which was chemically conjugated with rhEGF resulting in increased cellular proliferation of keratinocytes at the wounded area hence increasing the skin re-epithelization and also found to be effective in diabetic ulcers [95]. PCL and collagen were also combined and produced as electrospun nanofibers immobilized with epidermal growth factor which controlled the expression of a skin associated gene loricrin which was found to be promising composite in dermis revitalization [132].

Due to the oxygen permeability and barrier properties provided by the polyurethane (PU), it is concurrently used as a wound dressing. From the findings, it has been concluded that polyurethane is semipermeable in nature which can have extensive benefits in wound healing. Water permeability is also of prime importance from the wound to avoid the accumulation of water between the dressing and the wound to prevent the desiccation of wounds. Semipermeable dressings also suffer from the disadvantage of the accumulation of the fluid specifically after few days of dressings and hence wound aspiration is needed to prevent wound leaking and further infections [133, 134]. Waterborne polyurethane hydrogels were formulated and investigated on a full-length thickness rat model which resulted in the prevention of scab formation, keeping the wound area moist thereby completely closing the wound without any scar formation or healing marks [135]. Polyurethane foam wound dressing was developed and the technology was patented which resulted in enhanced wound closure as the dressing possessed moisture vapor transmission rate nearby to the ability of the surface of the wound to release serous exudate [136].

Polyethylene oxide is a high molecular weight, nonpolar, linear polymer having undesirable free energy which involves its interaction with proteins. PEO is a biocompatible polymer utilized in skin re-epithelization and in various other medical devices. For drug delivery, PEO is currently conjugated with biological proteins which displayed prolong circulation time [137]. Vigilon wound dressing was developed which consisted of 96% water and remaining polyethylene oxide and is nonadherent resulting in enhanced excellent wound healing as compared to other wound dressing making it a promising candidate for dermatological surgery [138]. Moreover, poly(hexamethylene biguanide) was fabricated with polyethylene oxide/chitosan electrospun nanofibers

[139]. Polyethylene oxide wound dressing was also fabricated with *Aloe vera* due to broad range of antimicrobial activity of *Aloe vera* as well as its effect on reducing the levels of prostaglandins and stimulating fibroblasts at the wounded site, which were investigated on the faces of the humans thereby displaying excellent and rapid closure of wounds [140]. Therefore, synthetic polymers play a major role in the healing of wound and skin re-epithelization and by further modifying the nanofibrous scaffold of the polymer such as by including drug or bioactive molecules, even better outcomes are achieved.

5. Conclusion

Although numerous therapies and treatment are available for treating wound healing, electrospun nanofibers has proven to be very effective in the rapid closure of wounds. Electrospinning-based formulation of nanofibers is long back technology for more than past five decades but from the past one decade, pace for dependence on nanofibers has been increased because of more exploration of its modified surface and properties.

There is a requirement of continuous monitoring of wounds, preventing the wound from getting more infected, keeping the wound area moist, enhancing the healing process, imitating extracellular matrix for the management of wound which is all achieved by the electrospun nanofibers but there is a need of further modification in the several parameters such as the selection of polymers, and bioactive molecules to be loaded onto the nanofibrous composites to scale-up and develop a cost-effective industrial process and to achieve even more outstanding wound healing properties. Electrospun fibers have the unique property of supporting various types of cells and further promoting the proliferation of cells. Nanofibers have mechanical properties improved in comparison to other nano-based delivery systems. Formulation scientists are still struggling for a diameter of fiber and its uniformity rather than the availability of several research proposals in relation to this. Fiber-related morphology are used to perform in the extracellular matrix. There is a strong need to develop designer electrospun nanofibers with clinical relevance and homogeneous distribution among the cells.

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CHAPTER 14

Nanomedicine in pulmonary delivery

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1. Nanomedicines and their pulmonary application

Nanomedicine can be defined as the application of nanobiotechnology to medicine, and the prefix nano in the word nanomedicine often refers to a billionth (1×10^{-9}) of a meter, which is the SI unit of length. A common consensus on the size scale is that at least one length scale of the nanotechnology device is below 100 nm. Nanomedicines in drug formulation and delivery are advantageous due to their unique characteristics exhibited by a small size in the nano range [3]. Therefore, nanomedicines are designed to enhance drug targeting and retention that helps to improve efficacy while reducing the side effects by controlling the biodistribution and release of the drug following administration. Nanoparticles for drug delivery incorporate a drug, either entrapped within a preferably biodegradable carrier matrix or shell, attached by covalent bonds or adsorbed to the surface of a particle or can be encased within a structure like a liposome or dendrimer [4–9]. Other drug delivery systems based on nanotechnology include polymeric nanoparticles, carbon nanotubes, quantum dots, gold nanoparticles, magnetic nanoparticles, polymeric micelles, and viral vectors [10]. Pure drug nanoparticles may also be synthesized. These nanosystems with distinct advantages also have clinical application as inhalation therapies and have been widely explored for their advantages as a component of inhaled formulations.

Delivery of nanomedicines through the pulmonary route can be intended for both local and systemic drug delivery though a large proportion of investigated nanoparticle formulations for inhalation are intended to treat local lung diseases. In pulmonary drug delivery, nanomedicines can be utilized to achieve improved drug solubility and lung deposition kinetics and controlled drug release in the lungs. In addition, nanoparticles offer a unique advantage in case of pulmonary delivery by avoiding their uptake by the macrophages due to their small size, which is important in minimizing loss of inhaled drugs to maximize the drug retention and bioavailability [1, 2]. On the other hand, their small size may make them prone to exhalation after pulmonary delivery if they are administered as nano-aerosol form rather than within microparticles. The appropriate aerodynamic size range for most efficient lung delivery falls between 1 and 5 μm [11] and, as

Theory and Applications of Nonparenteral Nanomedicines
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Table 1 Reasons to develop nanoparticles for pulmonary delivery.

Features of nanoparticles	Nanoparticle formulation	References
Enhanced solubility and dissolution	Liposomal nanoparticles	[12]
	Solid lipid nanoparticles	[13]
Enhanced lung retention	Polymeric nanoparticles	[14]
Uniform lung distribution	Polymeric nanoparticles	[15]
Reduction of drug toxicity	Solid lipid nanoparticles	[16, 17]
Cell-targeted drug delivery	Polymeric nanoparticles	[18]
	Solid lipid nanoparticles	[19]
Controlled drug release	Solid lipid nanoparticles	[20]
	Liposomal nanoparticles	[21]
Enhanced biological activity	Polymeric nanoparticles	[22]

such, nanomedicine formulation development for pulmonary delivery faces a unique challenge to carefully formulate nanoparticles into an inhalable formulation that, after inhalation, can deliver nanoparticles to desired sites in the lungs and exert their effect as nanosystems. The nanoparticles are then prepared into an inhalable form to achieve uniform lung distribution, specific cell-targeted delivery or controlled drug release etc. following inhalation, some of which are summarized in Table 1 and discussed throughout this chapter. Such nanomedicines are usually explored for their potential application in the treatment of local lung diseases such as asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF), lung cancer or tuberculosis, and sometimes for treatment of systemic diseases such as diabetes.

In the following sections, we discuss various nanoparticulate systems and their engineering approaches for delivery via the pulmonary route and discuss the unique advantages that nanomedicine has to offer for pulmonary delivery. The applications of such inhaled nanomedicines in the treatment of various diseases along with their challenges, toxicity issues, and future perspective of pulmonary delivery of nanomedicines are also discussed.

2. Advantages of pulmonary delivery of nanomedicines

2.1 Distribution of inhaled nanoparticle systems

Nanoparticles, as carriers for inhaled drugs, can lead to a uniform distribution within the airways and can have significant clinical implication [15, 23]. Nanoparticles can sometimes be incorporated on the lipid bilayer of cell membranes and can be translocated across the epithelial cells for intracellular pharmacologic effects as it exhibits the same size as other biological bodies [24].

2.2 Prolonged release after inhalation

Nanosystems may also act as controlled-release reservoirs, maintaining the therapeutic effect of the active pharmaceutical ingredient with prolonged duration while reducing the dose frequency, adverse effects, and eventually toxicity [18, 25]. Sustained drug release in the lungs is achieved as carriers used in nanosystems can escape rapid phagocytosis by macrophages in the lung and persist in the lung tissues for a long period. Carriers can also either be embedded or conjugated with microparticles or polymers, to achieve further controlled or tailored drug release [26].

2.3 Increased solubility and dissolution rate

Nanoparticles after pulmonary administration can also be designed to exhibit an increase in solubility and dissolution rate owing to their smaller particle size and larger surface area. Enhancement of solubility and dissolution rate can lead to enhanced bioavailability which allows a lower dose to be administered to achieve the same therapeutic effect, potentially also minimizing the adverse effects. Nanoparticle-based formulations also have the potential to enhance the bioavailability of poorly soluble drugs upon pulmonary delivery compared to the oral route of administration [27].

2.4 Inhaled delivery of macromolecules

Macromolecules exhibit highly hydrophilic nature and have large molecular sizes that are susceptible to intestinal enzyme degradation and show reduced permeability. Macromolecule-based nanoparticles for pulmonary delivery provide higher bioavailability and stability compared to their conventional analog. In recent years, macromolecules including insulin, human growth hormone, calcitonin, and interferon-alpha have been investigated for pulmonary administration [28].

2.5 Targeted drug delivery and internalization by cells following inhalation

Drug-loaded nanoparticles must overcome a number of physiological transport barriers to reach their specific site, usually the targeted pulmonary cells for local therapies. Efficient translocation of nanoparticles across the mucosal barrier in the lungs is a prerequisite for targeted drug delivery which can be achieved by appropriate surface engineering. Nanosystems such as polymeric nanoparticles can be modified with mucoadhesive and mucus-penetrative polymers to control the local distribution and bioavailability of inhaled nanoparticles [14]. Macrophages and dendritic cells are an integral part of the pulmonary system which modulates the innate immune system of the lung [29]. Hence, nanoparticle vaccine research is also of interest in providing optimal immune responses against respiratory disorders by allowing surface engineering of nanoparticles to incorporate specific ligands that help them reach the target immune cells. This may allow the

internalization of the nanoparticles by specific cells after inhalation. Another example of the application of inhaled nanoparticles in drug targeting and internalization by specific cells is in the case of antituberculosis (TB) therapy in which delivery of nanoparticles loaded with antibiotics to the infected macrophages site in the lungs is desired [30, 31]. Such active drug targeting to macrophages in anti-TB inhalation therapy has been reported to be possible by surface engineering of solid lipid nanoparticle assemblies by methyl α-D-mannopyranoside that recognize mannose receptors located on infected alveolar macrophages and facilitate cell internalization [32]. Targeting lung cancer cells is also an important application of inhaled nanoparticles which may be achieved through active tumor targeting (by receptor-mediated endocytosis) [33].

3. Considerations for pulmonary delivery of nanomedicines

3.1 Physicochemical considerations

3.1.1 Size and surface area

Nanoparticles are, of course, most readily defined and described by their size. As discussed in detail in subsequent sections, the influence of size on performance and response has been the primary physicochemical parameter used to evaluate nanoparticles. The size of nanoparticles may have a profound effect on how they act. For example, gold nanoparticles show intriguing size-toxicity dependency. Colloidal gold, above 13 nm, are regarded as nontoxic up to micromolar concentrations [34]. However, particles less than 2 nm have been shown to have high toxicity in a number of cell lines [35]. Molecular gold is also marketed in various products as an anti-inflammatory drug. Thus, there appears to be an intermediate size between molecular gold and nano-sized gold that results in unexpected toxicity.

In addition to the possible surprising effects of small changes in size, one should also consider the accompanying changes in surface area and volume (mass). As the diameter of particles is reduced, the surface area is markedly increased, such that it becomes quickly similar to the volume of the particle itself (Table 2). At larger scales, the volume, or mass

Table 2 Size, surface area, and volume relationships.

Diameter	Surface area	Volume	Surface area:volume ratio
5	79	65	1.2
10	314	524	0.6
25	1963	8181	0.24
50	7854	65,450	0.12
100	31,416	523,599	0.06
250	196,350	8,181,231	0.024
500	785,398	65,449,847	0.012
1000	3,141,593	523,598,776	0.006
10,000	314,159,265	523,598,775,598	0.0006

dominates the particle properties and this is reflected in many of the physicochemical observations and studies made at the micron and larger scales. The surface phenomenon may begin to dominate the behavior of particles when diameters approach the nanoscale. It should be evident to researchers in the inhalation aerosol field that surface phenomena are significant at the low micron scale (e.g., drug particle adhesion and cohesion in dry powder formulations) where Van der Waals forces begin to dominate over gravitational forces [36].

3.1.2 Shape and surface morphology

Nanoparticle shape has been less reported in fields of drug delivery. The shape can be controlled during the synthesis of nanoparticles, depending on the method [37]. The shape may also be important for mechanical strength as well as the biological effect [38]. Carbon-based nanomaterials such as fullerenes (single and multiwalled nanotubes, C_{60} and its analogues) have been studied as a family of nanoparticles and it is now clear that size, shape, surface charge, and chemical functionality are important for modulating cellular responses. Spherical C_{60} nanoparticles were not toxic but anisotropic nanotubes are toxic [34]. The well-known example of asbestos with high aspect ratio and high toxicity (chronic inflammation) compared to bulk silica that lacks these attributes illustrates the potential influence of shape [39].

The surface morphology of the particles also affects aerosolization by affecting particle agglomeration and deposition. Smooth surfaces are not preferred since they tend to increase the interaction between particles while rough or wrinkled surfaces tend to increase the aerosolization efficiency by decreasing the contact area between particles and reducing particle interactions [40].

3.1.3 Dissolution

Poorly soluble drugs are a ubiquitous problem seen in the pharmaceutical world, resulting in low bioavailability and/or distorted absorption. Not only does changing the size of a drug either alone or by incorporation with a particulate system for nanotechnology delivery help in increasing drug solubility, dissolution, and bioavailability, but it can also help with intracellular uptake and allow for avoidance of biological barriers to reach a site of interest. For pulmonary drug delivery applications, it may be speculated that delivery of poorly soluble compounds may lead to a very different disposition and clearance mechanisms when delivered as nanoparticles as compared to more traditional microparticles. Dissolution rates, interactions with macrophages, epithelial translocation, and other differences may lead to very different pharmacokinetics.

3.1.4 Drug release from nanoparticles

Although most inhaled products are designed for immediate release, there are a number of groups investigating the potential for controlled or sustained release into the lung. In many of these research programs, nanoparticles or nanostructured particles are specifically designed.

Drug release can be controlled and modulated using nanoparticles via a variety of mechanisms. For example, control of drug release can be obtained by matching nanoparticle size with polymer/hydrogel mesh size, by crosslinking nanoparticles to control matrix mesh size [41], using magnetically active nanoparticles to trigger drug release [42, 43], by controlling the size of nanoparticles, among a myriad of other options.

3.1.5 Aerosolization and delivery

For inhaled formulations, the aerodynamic diameter, which is the effective diameter of particles under the influence of airflow, is the most important parameter for their aerosolization efficiency. Depending on their aerodynamic diameter (D), distribution of particles along the respiratory tract takes place via three main mechanisms: inertial impaction ($D > 5\,\mu m$), gravitational sedimentation (D between 1 and $5\,\mu m$), and diffusion ($D < 1\,\mu m$) as shown in Table 3 [44]. The particles with an aerodynamic diameter larger than $5\,\mu m$ deposit in the upper airways while those with aerodynamic diameter between 1 and $5\,\mu m$ deposit in the lower airways (bronchioles and alveoli) making them suitable for deep lung deposition. On the other hand, particles with an aerodynamic diameter less than $1\,\mu m$ (usually nanoparticles) can be inhaled and are preferentially deposited in the deep lung, but most are readily exhaled [45] due to the deposition mechanism reliance on diffusion. It is important to note, however, that many nanoparticle systems designed for inhalation can be carried aerodynamically to deposit in the airways using microparticles or droplets. Bi-modal particle deposition occurs for particles within the nanometer range ($<1000\,nm$), where up to 80% of particles are exhaled [45]. In addition, efficient generation of nanoparticulate aerosols for drug delivery is limited due to the strong van der Waals forces that cause cohesion of particles of this size and also the sheer number of particles required to be produced to carry the same drug payload is daunting (see Table 2). Because of these hindrances to the delivery of nanoparticulate aerosols to the lung, microparticles encapsulating or carrying nanoparticles have been used to increase drug delivery, aerosol efficiency, protect the therapeutic agents, and to control nanoparticle distribution following deposition in the lung.

Table 3 Size-dependent deposition of inhaled particles within the respiratory tract.

Particle size (μm)	Delivery site	Mechanism of site delivery
5–9	Mouth, throat, large upper airways	Impaction
1–5	Bronchioles, smaller branched airways	Gravitational sedimentation
<0.5	Alveolar region, though readily exhaled	Brownian diffusion

From W. Yang, J.I. Peters, R.O. Williams III, Inhaled nanoparticles—a current review, Int. J. Pharm. 356 (1–2) (2008) 239–247.

3.2 Biological considerations

3.2.1 Permeation and cellular interactions

The different fates of nanoparticles after inhalation are represented in Fig. 1 [46], which shows the permeation, cellular interactions, and clearance pathways of nanoparticles in the lung. It is generally thought that nanoparticle transport across the lung epithelia can occur by three mechanisms: (1) passive diffusion either by transcellular, paracellular, or particle size-dependent diffusion; (2) carrier-mediated transport by receptor-mediated transport or efflux transporter proteins; and (3) vesicle-mediated endocytosis and transcytosis most likely by caveolae [47, 48].

Passive diffusion appears to be influenced by the size and lipophilicity of molecules. Particulates weighing <40 kDa are believed to passively diffuse through intercellular junction pores [47, 49]. For hydrophilic drugs, the molecular radius has an inverse effect on the rate of diffusion [47].

Carrier-mediated transport is modulated with specific receptor-ligand pairs to interact with the cell surface and trigger downstream signal pathways to internalize the particles. A candidate for mediated transport in type II epithelial cells is the peptide transporter PEPT2, a high-affinity peptide expressed on the apical membrane [47]. Nanoparticles may be coated with functional groups that are specific to certain receptors on the cell types of interest to best achieve internalization.

For epithelial type I cells, a high density of membrane vesicles have been known, with a predominance of noncoated or smooth-coated vesicle populations, known as caveolae

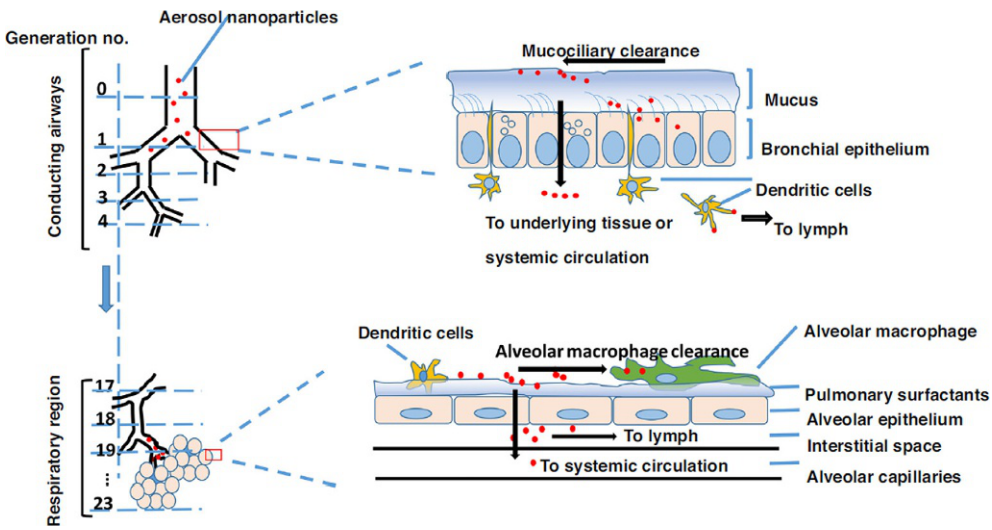

Fig. 1 Fate of nanoparticles after deposition in the lung. *Reproduced with permission from Q. Liu, et al., Physicochemical properties affecting the fate of nanoparticles in pulmonary drug delivery, Drug Discov. Today 25 (1) (2019) 150–159, https://doi.org/10.1016/j.drudis.2019.09.023.*

[50]. Caveolae are flask-like shaped invaginations 50–100 nm wide that are connected to the plasma membrane. It is generally accepted that vesicle-mediated trafficking is a minor pathway for protein absorption into the air-blood barrier for systemic exposure [47].

Alveolar regions of the lung contain four cell types: epithelial type I and II cells, alveolar brush cells (type III), and alveolar macrophages. Approximately 96% of the surface area contains type I cells with an average thickness of 0.26 μm, with nearly 3% of the surface being covered by type II cells [47]. These cell types are joined by tight junctions of varying tightness caused by the heterogeneous assortment of epithelial cells [47]. Moreover, the alveolar macrophages present a formidable barrier for pulmonary drug delivery in many cases. Macrophages are phagocytic immune cells whose primary purpose is to remove foreign particles and pathogens from the alveoli, with the ideal particle size for uptake being 1–3 μm [47]. By using nanoparticles to deliver drugs to the alveolar region, macrophage avoidance could be facilitated allowing for a longer particle to lung exposure [48, 51].

Nanoparticle pathways into epithelium cell layers of the lung are not well understood, despite these cells being the predominate type. Different nanoparticle modifications can be made to cause changes in cell internalization; size, surface charge/coating, and shape. Surface charge affects the permeability of particles into cells, with uncharged molecules being internalized with a lesser extent than cationic or anionic molecules [51]. Pulmonary epithelium seems to have a high permeability to compounds of the high molecular polar surface area [47]. Surface coatings or functionalities are used to stabilize particles as well as direct particles to specific ligands on cell surfaces of interest and affect cellular interactions [48, 52]. Size is also believed to affect cellular uptake of particles, with microparticles having a limited internalization compared to nanoparticles [48, 51, 52]. Furthermore, particles of 200–500 nm are taken in by clathrin-coated pits in melanoma cells [53]. The shape of particles may affect a cell's ability to internalize the particle by causing the vesicles of internalization to take on the shape of the particle, though little is known [52]. Ultimately, if nanoparticles can be designed to pass through the epithelium while containing the drug, then systemic delivery can be achieved. Furthermore, if such nanoparticles can be created to be taken up within the cells of the lung, then site-specific delivery would be facilitated with limited systemic toxicity.

3.2.2 Clearance of nanoparticles

Depending on the particle properties and their distribution, nanoparticles are cleared predominantly via three fundamental mechanisms: mucociliary clearance, phagocytosis, and systemic uptake. In the upper respiratory tract, mucociliary clearance is the primary mechanism. Mucus is produced by epithelial goblet cells and submucosal glands to form a thick viscoelastic hydrogel, up to 30 μm, largely comprised of glycoproteins and water. Inhaled particles which are trapped on, and in the mucus layer ascend about 3 mm per minute, toward the gastrointestinal tract by a metachronous beating of cilia and eliminated by coughing out or swallowing [54]. Mucociliary clearance is impaired in

pulmonary disorders such as CF, asthma, and COPD. For example, CF is characterized by normal ciliary structure and function but a secretion of thick mucus in the airways is present that is not cleared easily which may affect the clearance of inhaled drugs leading to altered bioavailability [55]. Particle size is equally important for the clearance of particles. Particles >5 μm in size, deposited in the upper and central respiratory tract is dominantly removed via mucociliary clearance. In the deeper lung, the mechanisms such as mucociliary clearance, phagocytosis, and endocytosis are present that are relatively depending on the dissolution profile of the particles. Phagocytosis by alveolar macrophages is considered to be the primary clearance mechanism. This process involves primary phagocytic cells for the innate immune system, engulfing and clearing exogenous materials from the respiratory tract. Phagocytosis by macrophages is predominantly responsible for the clearance of particles between 0.5 and 5 μm in size. Particle size with <200 nm is not recognized by macrophages due to their nanoscale size and can enhance rapid uptake by the epithelial cells [1, 2, 44].

3.2.3 Extracellular interactions

An additional biological consideration for nanoparticle delivery is the mucus barrier found within the conducting airways. The importance of this barrier in healthy and diseased state has been documented [56–60]. The mucus layer has a primary function of trapping exogenous substances until they can be removed by clearance mechanisms such as ciliary movement within the lungs [61]. An overall complex molecular composition and structure of viscous and elastic gel-like properties of glycoprotein mucins help maintain this protective mechanism. The general structure of mucus is thought to have a "bottle brush" configuration where oligosaccharide chains are attached to a protein core resulting in providing regions of electrostatic, hydrophobic, and hydrogen-bonding interactions to occur [56, 62, 63].

Nanoparticles have been noted to become entrapped within mucus. This is in part due to the structure and function of mucus, as well as the particle size and coating. In CF sputum studies, Sanders and group noticed that nanoparticle size played a significant role in nanoparticle transport [64]. The smaller the particle (~120 nm), the more likely the particle would be able to slip through the mesh spacing of the mucin protein strands. Though contradictory to this observation, it has been seen recently that larger particles (500 nm) could be modified to slip through cervical mucus by changing the surface chemistry of particles using 2 kDa polyethylene glycol (PEG) polymer [65]. Altogether, it seems that nanoparticles can be modified to limit interactions with mucus.

4. Nano-particulate systems for pulmonary drug delivery

4.1 Liposomes for pulmonary delivery

Liposomes are concentric bilayer vesicles in which an aqueous volume is entirely enclosed by a membranous lipid bilayer composed mainly of natural or synthetic

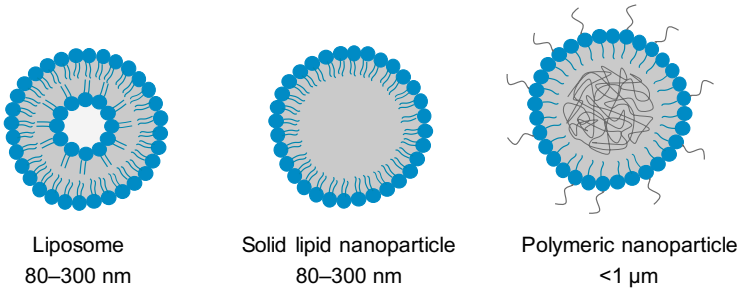

Fig. 2 Schematic diagrams of different nanoparticulate systems.

phospholipids which are biocompatible and degradable as represented in Fig. 2 [66, 67]. Due to their entrapping capabilities, liposomes have been considered for drug carriers since the late 1970s.

Due to their unique structure, liposomes can incorporate both hydrophilic and lipophilic drugs inside the aqueous core and within the bilayer, respectively [68]. For pulmonary drug delivery, liposomes are advantageous because they are reported to show good compatibility with lung surfactant components and cause low or negligible irritation to local lung tissue and can target specific epithelia using surface-bound ligands or antibodies which increases retention time and reduces toxicity. The most important parameters of liposomes to characterize their suitability for pulmonary delivery are their size, entrapment efficiency, drug loading, and stability. These factors may have to be measured to identify the most suitable preparation technique for each drug candidate for effective lung deposition.

4.1.1 Preparation of liposomes

Liposomes can be prepared either as dry powder liposomes or as liposomal suspensions. Liposomes spontaneously form upon the dispersion of phospholipids in an aqueous medium [69, 70]. This spontaneous formation is due in part to the hydrophilic interactions of the head groups of the lipid with water causing multilamellar and vesicles (unilamellar) systems to be created. Commonly, liposomal dry powders for pulmonary delivery are prepared by freeze-drying, spray-drying, and spray freeze drying, after which, the liposomes containing drug molecules can be delivered using inhalers [66]. On the other hand, liposomal solutions can also be used for pulmonary delivery by preparing the aerosols of the liquid form using devices such as a medical nebulizer, pressurized metered-dose inhalers or soft mist inhalers. The examples of drug candidates and the formulation features of liposomes intended for pulmonary delivery are summarized in Table 4.

Table 4 Drug candidates studied for liposomal formulation for inhaled delivery.

Drug candidate	Preparation of liposomes	Formulation features	Reference
Calcitonin	Hydration method followed by extrusion	Improvement in liposome-cell interaction and bioadhesion to lung epithelia.	[71]
Oseltamivir phosphate	Film dispersion followed by hydration	Nonaggregating spherical particles with reduced particle-particle cohesion.	[72]
Ciprofloxacin	Lipid film hydration	In vitro evaluation demonstrated a sustained release pattern.	[21]
Gemcitabine	Emulsification solvent evaporation	Improved lung deposition of liposomal formulation.	[73]
Ciprofloxacin	Extrusion and solvent removal	Good inhalation aerosol delivery and controlled drug release.	[74]

4.2 Solid lipid nanoparticles for pulmonary delivery

In the 1980s, Speiser and coworkers began to develop the use of solid lipids instead of liquid oils to achieve controlled drug release by encapsulation within a solid lipid core of an emulsion [75–77]. Solid lipid nanoparticles (SLN) are aqueous colloidal dispersions, comprising of solid biodegradable lipids in the matrix which are, in body and room temperature, different from liquid cores of liposomes and emulsions as represented by schematic diagram in Fig. 2 [16, 78, 79]. SLN are composed of lipids dispersed in an aqueous solution of surfactants (e.g., phospholipids), which help the mixing of inner lipid phase with the external water phase to give SLN dispersions [17, 79].

SLN are advantageous because they are generally well-tolerated due to their biocompatibility and provide controlled release of drug molecules and also because of their feasibility for delivery of both lipophilic and hydrophilic drugs. Moreover, SLN do not require the use of organic solvents during formulation and are more stable than emulsions and liposomal formulations, less toxic, and offer easy cost-effective large-scale production using high-pressure homogenization [17]. Due to their small diameter and surface characteristics, suitability either for therapeutic purposes or for cancer metastasis visualization upon pulmonary delivery can be expected for these particles [80]. A controlled release could be obtained by limiting the drug mobility within the solid lipid.

4.2.1 Preparation of solid lipid-nanoparticles

Speiser and coworkers created microparticles and "nanopellets" by spray drying techniques, high-shear mixing or ultrasound dispersion. Through the advancement of lipid

particles, it was found that high-pressure homogenization was a more effective method for producing submicron-sized dispersions compared to previous techniques [81, 82].

In addition to high-shear hot homogenization, SLNs can also be prepared using several other methods namely, high shear cold homogenization, ultrasonication, microemulsion-based SLN preparation, solvent evaporation, and spray drying. In high-shear hot homogenization, the lipid phase is melted at a temperature above its melting point and a hot aqueous phase consisting surfactant and emulsifier in purified water is added to the lipid phase and homogenized using a high-shear mixer. The cooling of this emulsion leads to crystallization of the lipid and the formation of SLNs, useful for the formulation of sensitive drug molecules [83]. Whereas in high-shear cold homogenization method, the drug is combined into melted lipid; the melted lipid is cooled rapidly using liquid nitrogen or dry ice. The solid material is then grounded using a mortar mill. Thus prepared lipid microparticles are then dispersed in a cold emulsifier solution at or below room temperature. In comparison to the hot homogenization, the particle size is larger and the size distribution is broader [79].

Ultrasonication method is also called high-speed homogenization and uses ultrasound waves to prepare nanoparticles. The main advantage of this method is that it produces smaller particles with reduced polydispersity and aggregation of nanoparticles. However, this technique is also associated with the disadvantages of potential metal contaminations and physical instability like particle growth upon storage [16, 79].

Microemulsion-based SLN preparation is based on the dilution of microemulsions prepared by stirring a transparent mixture of low melting fatty acid, emulsifier, co-emulsifiers, and water at a temperature of 65–70°C. During stirring, the hot microemulsion is dispersed in cold water (2–3°C) [79].

In the solvent evaporation technique, the lipophilic material is dissolved in a water-immiscible organic solvent (such as cyclohexane) that is emulsified in an aqueous phase. As the solvent evaporates, nanoparticle dispersion can be formed by the precipitation of the lipid in the aqueous medium.

Spray drying, a solvent evaporation technique, is utilized to prepare powder formulations of SLN. This technique transforms an aqueous SLN dispersion into a powder product. It is cost-effective compared to lyophilization. This method may cause particle aggregation due to high temperature, shear forces, and partial melting of the particle, and therefore, the use of lipid with melting point >70°C is recommended for preparation by this technique. The examples of SLN-based formulations investigated for pulmonary delivery are summarized in Table 5.

4.3 Polymeric nanoparticles-based formulations for pulmonary delivery

Polymeric nanoparticles are small spherical particles with a diameter of around 10–1000 nm and are comprised of macromolecular, polymeric materials that encapsulate

Table 5 Example of drug loaded SLN based formulations for pulmonary delivery.

Drug candidate	Preparation method	Formulation features	Reference
Model protein (Papain, PAP)	Hot high-shear homogenization	Simple, continuous, easily scaled, and cost effective.	[83]
Budesonide	Ultrasonication and homogenization	Deep lung deposition with prolonged release, low toxicity.	[16]
Erlotinib	Hot homogenization	Rapid drug deposition in lungs, less systemic side effects.	[17]
Quorum sensing inhibitors	Hot melt homogenization	Improved drug loading and mucus penetrative property.	[84]
Naringenin	Emulsification and low-temperature solidification	Sustained drug release, improved bioavailability.	[20]
Sildenafil	High pressure hot melt homogenization	Gradual increase in particle size of sildenafil-loaded SLN over time.	[85]
Ketoprofen and Indomethacin	High pressure homogenization	A loading capacity achieved higher than the thermodynamic limit of the solubility of the drugs in molten lipids was achieved.	[13]
Levofloxacin	Ultrasonication	Sustained drug release but low drug encapsulation.	[86]
Insulin	Reverse micelle-double emulsion technique	Bioavailability of pulmonary SLN was comparable to that from intravenous route.	[87]
Baicalein	Solvent-injection method	Mucus-penetrative nanoparticles exhibited superior mucus penetration, local distribution and bioavailability compared to mucoadhesive nanoparticles.	[14]
Rapamycin	Hot-evaporation technique	Sustained release profile suitable for entry into the lymphatic system.	[88]

or dissolve with the drug which can be administered with a liquid carrier in a solution form or in a dry powder form [89]. Polymeric nanoparticles have been shown to improve the therapeutic index by modulating both the pharmacokinetic and pharmacodynamic properties of drugs [89]. Many other therapeutic effects such as biodistribution, in vivo stability, bioavailability, permeability through biological barriers, and drug release properties have been found to be improved by the use of nanoparticles [89]. These features of polymeric nanoparticles provide a vast potential for pulmonary drug delivery of drugs efficiently to treat local and systemic diseases [90].

4.3.1 Preparation of polymeric nanoparticles

Polymeric nanoparticles can be prepared using various methods such as solvent-evaporation (spray-drying), freeze-drying, spray freeze drying, emulsion diffusion, and antisolvent precipitation. Common polymeric agents used to prepare nanoparticles for pulmonary delivery include poly(lactic-*co*-glycolic acid) (PLGA), poly(vinyl alcohol) (PVA), polyvinylpyrrolidone (PVP), dipalmitoylphosphatidylcholine (DPPC), and dipalmitoylphosphatidylgylcerol (DPPG) [89]. Drug loading can be achieved either by incorporation of the drug at the time of nanoparticle production or by adsorbing the drug after the formulation of nanoparticles by incubating them in the drug solution [91]. Once the drug is loaded onto nanoparticles, the release rate will depend on desorption of the surface-bound/adsorbed drug, diffusion through the nanoparticle matrix, diffusion through the polymer wall, matrix erosion, and a combined erosion process [92]. A transformation process is required to use nanoparticles for inhalation therapy. This process involves co-spray drying with thermal-protectant which turns nanoparticles into powders, resulting in microspheres with adequate aerodynamic properties. These properties provide a potential application for lung delivery as a solid formulation [92]. Examples of polymeric nanoparticles based drug formulations explored for pulmonary delivery are summarized in Table 6.

Table 6 Examples of polymeric nanoparticle system for pulmonary drug delivery.

Drug candidate	Preparation method (polymers)	Formulation features	Reference
DNA and erythropoietin	Gelatin, chitosan, alginate, PLGA, PLGA-chitosan, and PLGA-poly(ethylene glycol) nanoparticles	Encapsulation of plasmid DNA and erythropoietin in gelatin and PLGA.	[90]
Fluticasone propionate	Poly(lactide)-based mucus penetrating and mucoadhesive particles	Preparation of mucus-penetrating and mucoadhesive particles.	[93]
pDNA	Nanoparticles of PLGA and polyethylenimine	Decreased toxicity, enhanced cellular uptake, improved aerosolization	[94]
Ethionamide	Carrageenan-stabilized chitosan alginate nanoparticles	Enhanced stability of the nanoparticles and entrapment of ethionamide in the nanoparticles.	[95]
Ciprofloxacin	PLGA-based nanoparticles	In vitro anti-biofilm activity against mature *P. aeruginosa* and *S. aureus*.	[22]
Rifampicin	Drug loaded into trimethyl chitosan chloride glycerosomes	Increased mean diameter of particles, reduced in vitro drug toxicity and increased efficacy.	[96]

4.4 Other nanocarriers

4.4.1 Effervescent nanoparticles

Effervescent technology is favorably used for oral formulations for vitamin supplements, analgesics, and dyspepsia. However, recent studies have emerged for alternative use in pulmonary drug delivery to enhance drug dispersion. The effervescent nanoparticle is an emerging technology for inhalable dry powders. The main mechanism of effervescent technology is the release of gas bubbles leading to an increase in drug dissolution and enhanced dispersion of nanoparticles from the dry carrier particles [97]. The effervescent formulations commonly add sodium carbonate and citric acid which lead to the formation of gas when in association with water molecules consequently increasing the volume during the transition phase from solid to gas, thereby achieving a rapid onset. Ammonium, ammonium salts, or buffer solution are also used to maintain the pH of the solution to impede an effervescent reaction preceding to manufacturing process [98]. Studies have shown that a wide distribution and a deep lung delivery can be achieved by incorporating drug-loaded effervescent nanoparticles in a mouse model [99]. In a study conducted by Jyoti et al., inhalable nanoparticles containing 9-bromo-noscapine, an anticancer agent showed a rapid dispersion and enhanced aerosolization of effervescent carriers compared to noneffervescent carriers leading to significant antitumor action [100]. Ely et al. investigated the release and dissolution of ciprofloxacin-loaded effervescent carrier compared to conventional lactose based carrier. The effervescent carrier release was found to be almost twice as the conventional carrier. The energetic release mechanism of effervescent technology provides a suitable particle size for deep lung delivery and avoids agglomeration [98]. While further safety trials can be investigated, the effervescent nanoparticle is one outset for treating pulmonary diseases with noninvasive methods.

4.4.2 Metallic nanoparticles

Metallic nanoparticles have elicited much interest in biomedical applications. This can be used in the field of drug delivery and imaging. A magnetic metal can be useful since the application of a magnetic field can concentrate the particles in a desired particular region which can ultimately be utilized in targeted delivery. The high intensity of a magnetic field can also be used for targeted cell death. For example, simple superparamagnetic iron-oxide nanoparticles or Fe_3O_4-coated hybrid of polymer or lipid-coated nanoparticles can be used in the treatment of lung cancer. A good example of the application of such nanoparticulate system is reported for cisplatin, an anticancer agent, incorporated with a complex coated Fe_3O_4 with PLGA-PEG copolymer which was found to show an improved antitumor activity of cisplatin in lung cancer [101]. It is also well reported that some metals are capable of causing potential cytotoxicity. Gaihre et al. reported the increase in toxicity of doxorubicin-metallic nanoparticles in HeLa cells [102]. However, iron-oxide nanoparticles conjugated with gold improve the bioavailability

of nanocarriers and was considered safe for treating lung cancers [103]. Because of the aggregating nature of gold, gadolinium, and platinum at the tumor site, nanoparticles of these elements are also used for radiotherapy as well as other diagnostic purposes [104].

5. Modified nanoparticulate systems for delivery by inhalation

Apart from their extensive investigation in drug delivery as a whole, nanoparticles are also gaining popularity in inhalation drug delivery and are proving to be effective in the delivery of drugs as a component of the dry powder inhalers. Although nanoparticles have their distinct features and advantages as drug carriers or delivery systems, they face unique challenges in pulmonary drug delivery owing to their nano size which makes aerosolization of the nanoparticles, in their original shape and size, more difficult. Therefore, further modification of such nanoparticles is necessary to make them inhalable which mostly include preparing them into microparticles for efficient aerosolization. As a result, there are pulmonary drug delivery systems such as nano-in-micro particles, surface-modified nanoparticles, and nanoaggregates which are systems composed primarily of nanoparticles but are rendered inhalable after modification. Such systems are more likely to be advantageous than the conventional micro or nanoparticles in delivering certain category of drugs (such as drugs with very low or very high solubility) in a controlled manner to the desired site in the lungs. Nanoparticulate systems are also known to improve pharmacokinetic and pharmacodynamics of the loaded drug by allowing modification in the biodistribution, stability, bioavailability, and permeability of the drug [89]. Nanoparticles, however, in pulmonary drug delivery, are known to be associated with issues such as unwanted exhalation due to small size and low inertia, uninhibited aggregation and stability that may hinder efficient aerosolization [89, 105–107]. Therefore, the most challenging part with nanoparticles in pulmonary drug delivery and formulation is to maintain the critical physicochemical parameters for aerosolization efficiency and thus successful inhalation [108]. This section discusses the techniques used to resolve the issues of nanoparticles in pulmonary drug delivery such as transforming nanoparticles into inhalable microparticulate systems or aggregates using different formulation techniques and particle engineering. Fig. 3 represents the different approaches to prepare nanoparticles into the inhalable formulation.

5.1 Surface modification of nanoparticles

Surface modification of nanoparticles refers to an alteration of physicochemical or biological characteristics of the surface of nanoparticles to modify their physicochemical properties, charge, size, hydrophobicity, and biocompatibility. Such modification can be achieved using various strategies that may include the introduction of chemical functional groups to the surface by covalent modification or by adsorption of biologically

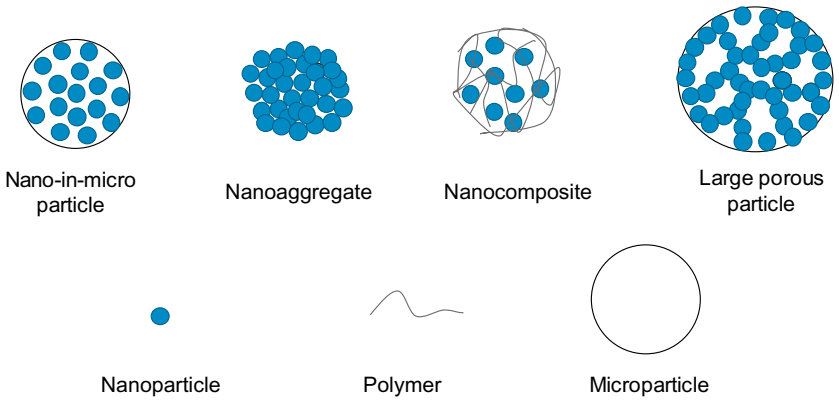

Fig. 3 Schematic diagram of different approaches to preparing nanoparticles into inhalable particles.

active molecules, such as proteins, surfactants, enzymes, antibodies, nucleic acids by noncovalent modification [109]. For modifications intended to improve aerosolization parameters of nanoparticles in pulmonary drug delivery, the noncovalent modification is more common while the covalent modification is more common in strategies intended to alter the tissue-binding and nanoparticle cellular uptake after inhalation [109, 110].

A simple and common surface modification of nanoparticles to improve aerosolization of nanoparticles involves the use of surface-active agents such as leucine. Leucine has been reported to be more effective than trehalose, sucrose, lactose, and mannitol in the stabilization of SLN [111]. A surface-active agent such as leucine can be used to reconstitute nanoparticles by spray-drying and stabilize their aerodynamic characteristics. The improvement in the aerosolization of such particles is driven by leucine induced surface modification of nanoparticles, which are usually irregular and aggregated, into corrugated particles that are more suitable from an aerodynamics point of view [111]. In a study that investigated the surface modification of nanoparticles of PVP using lactose microcarrier, various mechanisms such as surface adsorption, pore immersion, microaggregation, and encapsulation were identified for interaction between lactose and the nanoparticles depending on the type of lactose microcarrier [112]. The modification of nanoparticles using lactose was successful in improving the in vitro aerosolization efficiency of nanoparticles for efficient lung delivery. Use of lactose in combination with PEG 3000 has also been reported to be useful in improving inhalation performance of chitosan nanoparticles. Spray-dried chitosan nanoparticles when physically blended with spray-dried microparticles of lactose—PEG 3000, were found to show larger size attributes with reduced interparticulate aggregation which contributed in the improvement of inhalation performance of the particles [113]. Apart from modifications intended for improving aerosolization or the aerodynamic properties of the nanoparticles, surface

modification to alter mucus retention and penetration postinhalation has also been reported. Mucoadhesive and mucus-penetrative polymers were used for surface modification of polymeric nanoparticles loaded with a baicalein-phospholipid complex which yielded modified nanoparticles which showed higher diffusion rate and deeper penetration across the mucus layer, enhanced in vitro cellular uptake, increased drug distribution in airways, and superior local distribution and bioavailability of baicalein [14].

5.2 Nano-in-micro systems for inhaled delivery

Nanoparticulate systems for pulmonary drug delivery show promise in widening the applications of inhalable formulations while at the same time being limited by its own key feature—the small, nano-range particle size. Since the diameter of particles for inhalation is desired to be between 1 and 5 μm, nanoparticles fall below the minimum size for the particles which limit their capability to be delivered and retained at the deep lung sites. Formulation approaches in pulmonary drug delivery, therefore, focus on the formulation of such nanoparticles into micron-sized particles which led to the terminology called "nano-in-micro particles." Nano-in-micro systems refer to the incorporation of nanoparticles into a larger microparticle system which acts as the carrier of the drug-loaded nanosystems and facilitates their delivery into the deep lung by improving aerosolization efficiency.

Preparation of nano-in-micro systems requires two steps that involve the preparation of the nanoparticles first, followed by the incorporation of nanoparticles into a microparticulate system. Since these types of systems are still under development and research, the most commonly reported method of preparation of nano-in-micro formulations for pulmonary delivery has been the spray drying method as the basic technique [114–118]. The applications of such nano-in-micro formulations include delivery of antibodies, chemotherapeutics as well as agents for the theragnosis of lung cancer, all of them appropriate for delivery via the lungs. A system for pulmonary delivery of antibodies was reported as simultaneously manufactured nano-in-micro (SIMANIM) particles prepared by spray-drying of a double-emulsion containing human immunoglobulin (IgG), lactose, PLGA, and DPPC [115]. The end product was a powder of microparticles with size adequate for inhalation; however, the particles upon contact with aqueous media were able to be partially dissolved to form nanoparticles which were about 10-fold smaller than their original diameter. Another notable example of a nano-in-micro system for pulmonary delivery is the incorporation of functionalized gold nanoparticles into respirable microparticles [117]. The functionalized nanoparticles were incorporated into a chitosan matrix by supercritical CO_2-assisted spray-drying that yielded nano-in-micro clean ultrafine dry powder formulation with particle aerodynamic sizes ranging between 3.2 and 3.8 μm and fine particle fraction (FPF) of 47%.

5.3 Nanoaggregates, large porous particles, and hollow nanocarriers

Several other approaches to formulate nanoparticles into inhalable particles include the preparation of nanoaggregates, large porous particles, and hollow nanocarriers which further manipulate the nanoparticle system-based formulation into a practically inhalable formulation suitable for lung delivery. Nanoaggregates are made up of agglomeration of nanoparticles that aggregate via physical van der Waals forces during preparation step such as spray-drying, that forms a crust of nanoaggregate layers resulting from self-assembly of nanoparticles due to evaporation of solvent at the interface of the droplet surface [33]. A notable example of nanoaggregates for dry powder inhalation is that of voriconazole produced by thin-film freezing method [119]. The thin-film freezing method was used to prepare a high potency dry powder inhalable formulation composed of voriconazole nanoaggregates which was aided by nanoparticles of mannitol on the surface of voriconazole nanoaggregates to enhance the aerosolization properties of the powder by modifying the surface texture. During the preparation of nanoaggregates for inhalation, several critical parameters such as solvent system composition, processing temperature, solid loading, and scale are considered important [119]. Porous nanoparticles aggregates particles (PNAP), the micron-sized peptide-containing nano-assemblies are another example of nanoaggregate system where nanoparticles are assembled to form a micron-sized particle, suitable for inhalation [120]. Similar to other systems, the micron size (1–5 μm) of the PNAP is important for aerodynamic properties and aerosolization while the nanoparticles (~300 nm) efficiently entrap peptides and facilitates dispersion in lung lumen after delivery. Preparation of such nanoaggregate systems is, therefore, advantageous in both efficient lung delivery as well as efficient accumulation into lungs, macrophage targeting and controlled drug release.

Among nanoaggregates, large porous particles, and hollow nanocarriers, the large porous particle approach is more popular in dry powder formulations intended for inhalation. Large porous particles were first reported as particles characterized by their large geometric size (>5 μm) and porous structure with mass densities around 0.1 g/cm^3 or less, designed to be efficiently delivered into deep lungs and to avoid the lungs' natural clearance mechanisms until the inhaled particles delivered their therapeutic payload [121, 122]. Large porous particle approach has been used to formulate several drugs for pulmonary delivery among which budesonide, meloxicam, curcumin, rifampicin, dexamethasone palmitate, heparin, and meloxicam are some examples [123–128].

Apart from these conventional large porous particle systems, another similar system of large porous particle comprised of adsorbed nanoparticles that can combine the advantage of large porous particles and nanoparticles while avoiding their limitations, have been reported [122]. Such systems can be prepared by spray-drying to form large porous particles comprised of nanoparticles that are held together by physical means, such as Van der

Waals forces, or within a matrix of other ingredients such as biopolymers or phospholipids. Once deposited in the lungs, such systems disassociate to yield nanoparticles that are loaded with the drugs.

There are similar other approaches reported in the literature often regarded as hollow nanocarriers. Hollow nanocarriers differ from porous particles in their preparation technique in which they are first created as solid particles with a core composed of a material such as silica, which is then removed in the subsequent step of preparation to obtain hollow particles. For example, a PEGylated paramagnetic hollow-nanosphere for pulmonary co-delivery of doxorubicin and methotrexate was prepared in two steps [114]. The first step involved the synthesis of magnetic-silica nanoparticles and their coating with amino/acrylate groups and derivatization with PEG. In the second step, silica was removed to provide hollow structured nanoparticles. The nanoparticles were finally dispersed in an aqueous solution of mannitol to obtain dry powders of nanoparticles within microspheres. Hollow nanocarriers and nanoaggregates may, therefore, have advantages of efficient lung delivery features for inhalation and controlled drug release from the nanosystems after inhalation.

6. Applications of inhaled nanomedicines

6.1 Treatment of respiratory and systemic diseases with inhaled nanomedicines

6.1.1 Asthma

Asthma is a major respiratory disease characterized by chronic inflammation conditions and mucus hyperproduction of the airways associated with hyperresponsiveness [129]. It is characterized by airway bronchodilator reversibility that differentiates it from other irreversible obstructive lung diseases [130, 131]. The current inhaled therapy for the management of asthma is dependent on inhaled delivery of drugs as dry powder inhalers, metered-dose inhalers, or nebulizers. While inhaled therapy based on microparticles have been used clinically over the years, nanoparticles-based formulations have recently been investigated for newer drug candidates with better therapeutic effects. For example, nanoparticle-based therapy for drugs such as andrographolide and baicalein was found to be promising for improved treatment outcomes [14, 132]. In the case of andrographolide, the administration of the nanoparticles from the inhaled route was found to be more efficacious than the oral route in a murine asthma model [132]. Similarly, for baicalein, the efficiency of inhaled therapy was improved by promoting mucus penetration with the help of nanoparticles based formulation. Therefore, for inflammatory lung diseases such as asthma, nanoparticles are recently under focus from a formulation point of view and are promising candidates to be employed to treat mucus hypersecretion and severe inflammation.

6.1.2 Chronic obstructive pulmonary disease

COPD is a common, noncommunicable, and preventable respiratory disease characterized by perpetual airflow limitation which is usually known to be a result of significant exposure to noxious particles or gases. The pathophysiology of COPD is characterized by small airway diseases which are mucosal inflammation and fibrosis (chronic obstructive bronchiolitis) and disruption of lung parenchyma (emphysema) [133]. Pharmacological therapy of COPD is similar to asthma with microparticles-based inhaled formulations delivered either as dry powder inhalers, metered dose inhalers or nebulization being the mainstay of clinical therapy. Nanomedicines of several drugs have now been investigated for improved therapy against COPD via the pulmonary route. Recent examples include the investigation of micro ribonucleic acids (miRNAs) [134, 135] and salmeterol xinafoate [136] as a part of the nanoparticles-based system for their pulmonary delivery in COPD management. The nanoparticulate formulation in such cases enabled the preparation of particles with good aerosolization parameters while retaining biological activities of the loaded molecules and achieving better retention in lung epithelium due to mucoadhesive properties of the nanoparticles.

6.1.3 Cystic fibrosis

CF is a heterogeneous recessive genetic disorder commonly found in the Caucasian population [137]. CF is caused by the mutation of CFTR (cystic fibrosis transmembrane conductance regulator) protein. In the lungs, mutation of CFTR protein leads to abnormal fluid flow across the epithelial membranes which results in thick mucus production making them prone to infections by bacteria such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* which ultimately leads to respiratory failure. Currently, treatment against CF is aimed to address the symptoms while there are no cures to stop the disease progression. Nanomedicine is considered as the promising approach to drug delivery against CF to improve the therapeutic efficacy by improving drug retention and controlled release after inhalation. Nanostructured lipid carriers loaded drugs have been found to be able to restore the expression and function of CFTR protein when delivered via inhalation and were highly effective in treating lung manifestations of CF [138]. Similarly, the potential of nanoparticles based inhaled systems for lung infection therapy in CF have been investigated and the nanoparticulate systems have shown promise with superior activity against lung infections in CF compared to the conventional drug delivery systems [139–141]. For example, Pilcer et al. produced co-spray-dried tobramycin nanoparticles-clarithromycin inhaled particulate system for the treatment of CF showed increased drug dispersion and improved aerosolization compared to physical blends [139]. On the other hand, Günday Türeli et al. engineered ciprofloxacin-loaded PLGA nanoparticles which showed increased antibacterial activity against *P. aeruginosa* infections in CF probably due to increased penetration through the thick mucus of CF [141].

6.1.4 Lung cancer

Lung cancer is the second most common cancer in both men and women worldwide. Approaches in inhaled formulation development against lung cancer have employed nanoparticle-based systems to enhance the drug bioavailability and stability as well as to improve anticancer drug retention at the target sites and to minimize cytotoxicity to healthy cells [142–145]. Enhanced anticancer activity in the lung tissue was demonstrated using inhaled nanoparticle formulation compared to the intravenous administration, which was achieved by conjugating anticancer agent, doxorubicin with PEGylated polylysine dendrimer [142]. The inhaled nanoparticles of doxorubicin-loaded dendrimer achieved >95% reduction of lung tumor burden compared to 30%–50% reduction achieved by intravenous administration. Sadhukha et al. investigated the use of novel, noninvasive nanoparticles for tumor ablation around an external magnetic field [144]. The results showed enhanced tumor retention and significant inhibition of in vivo lung tumor growth in a mouse cancer model.

6.1.5 Tuberculosis

TB is an infectious disease caused by bacillus *Mycobacterium tuberculosis*, the second leading cause of death through a single infection agent globally. Inhaled delivery of drugs is believed to be the mainstay to treat TB infection because the lungs are the primary site of entry of bacteria and they mostly reside within the caseating lesions of the lungs in pulmonary TB [146]. Moreover, inhaled drugs can also reach the systemic circulation for the treatment of extrapulmonary TB. In TB treatment, nanoparticles loaded with drugs can be utilized as a novel host-directed therapy for multidrug-resistant TB [147] or with appropriate surface engineering can be utilized for active targeting to macrophages in anti-TB inhalation therapy [32]. Similarly, inhalable nanoparticles can also serve as ideal carriers for the controlled release of anti-TB drugs [148].

6.1.6 Diabetes

Pulmonary delivery of insulin is the most important and common example of the application of the inhalation route in the treatment of systemic disease. Inhalation of insulin avoids the problem of degradation within the gastrointestinal tract and can be efficiently absorbed into the bloodstream through the lungs eliminating the need for subcutaneous injection, which is the mainstay of insulin delivery technique. A remarkable success with inhaled insulin is highlighted by the clinical approval of two dry powder inhaler systems of insulin among several formulations that were able to reach the clinical studies [149]. For pulmonary delivery of insulin, nanoparticle-based formulations are considered advantageous since they assure both stability and suitability for aerosolization for effective diabetic therapy [150, 151].

6.2 Pulmonary delivery of diagnostics

In recent years, nanomedicines have witnessed considerable development in the field of biomedical diagnostics and imaging. Unique properties of nanoparticles may be used in pulmonary X-rays diagnostics. In a study, gold nanoparticles assay was used for the early determination with active pulmonary TB compared to its conventional diagnostics. The conventional diagnostics involved the sputum smear microscopy and chest X-ray. The results showed that the sensitivity and specificity of TB nanogold assay were 95% and 100%, respectively. This proof-of-concept shows that nanogold assay was more accurate and faster than the conventional diagnostics in detecting active pulmonary TB [152]. Nanoparticles are also reported to boost magnetic resonance imaging (MRI) diagnostics of lung parenchyma [153]. In a study that investigated the pharmacokinetics of intratracheal administration of Gadolinium-DOTA nanoparticles in BALB/c female mice using ultrashort-echo-time proton MRI, signal enhancements were recorded of different organs including the lungs at different time points. It was observed that the nanoparticles passed from the lungs to the blood, then to the kidneys, and lastly to the bladder while it also showed no liver signal enhancements [153].

6.3 Therapeutic classes of inhaled nanomedicines

6.3.1 Small molecules

Low molecular weight molecules can be delivered to the lung allowing for rapid and efficient absorption. Lipid-soluble molecules are absorbed presumably by the lipid bilayer via the transcellular pathway. In contrast, lipid-insoluble molecules are likely to be transported by the paracellular route through the tight junctions. Tight junction transport takes into account the molecular weight and ionization of a molecule to allow for rates at which the molecule can pass [154]. To provide additional mechanisms of delivery nanoparticles have been used to carry low molecular weight drugs to a site of action [155].

6.3.2 Peptides and proteins

Systemic delivery of peptides and proteins can be achieved via the lung. The lung provides an ideal route for such delivery since it has a large surface area of $\sim 100\,m^2$ allowing for rapid absorption within the blood vessel rich alveolar regions, considered less invasive than parenteral administration, and contains low levels of enzymatic activity in comparison to the gastrointestinal tract. Further absorption systemically may be achieved by delivery to the central region of the lung through active transport processes observed in the cell types of the conducting airways, compared to the more passive or paracellular routes of transport within the alveoli. Additionally, the lung provides a relatively thin path for the diffusion of molecules to the bloodstream due to the rich supply of capillary vessels lining the alveolar regions of the lung. Formulation considerations for peptides and proteins will undoubtedly need to be considered. Naturally occurring peptides

are hydrophilic in nature making them not ideal candidates for delivery unless structural modifications or entrapment within particles occur. Despite any formulation issues, pulmonary delivery of metabolic hormones such as insulin, calcitonin, somatostatin, and growth hormones have been investigated for use in both humans and animals [156, 157].

Insulin is considered the most widely investigated protein for pulmonary delivery. In 1925, Gansslen performed the first study of the efficacy of insulin in humans after pulmonary delivery, claiming that reduced blood glucose levels occurred within 2.5 hours. In January 2006, the FDA approved the first inhaled insulin, Exubera from Pfizer Inc. By October 2007, Exubera was dropped, citing limited acceptance of both patients and physicians [157–159]. Insulin was again marketed as dry powder inhalers by Mannkind in 2012.

Despite the complications caused by Exubera, a continued interest in insulin and other proteins delivery (e.g., cyclosporine A) to the lung via nanoparticle systems has occurred at the clinical level [160, 161]. With over a decade of research, only a limited amount of kinetic work has been supplied, along with no guidance on criteria for the design of lipid carriers mainly due to the variation in formulation and evaluation methods. The data that has been provided concludes that in vitro release can be prolonged and that sustained in vivo effects are achievable for various proteins and that the main factors are the proteins, overall particle size, matrix composition, used surfactants, preparation and production methods, and the distribution of the protein within the matrix. For example, with oil and water emulsion techniques, a burst release may be observed due to the hydrophilic nature of the peptides and proteins which accumulate at the oil-water interface during preparation [162]. While in core-shell incorporation methods, such as used by Schwarz et al., a drug shell is formed due to the accumulation of protein at the surface of the SLN causing modified release rates that can be considered undesirable unless one is trying to achieve an initial dose with a limited slow release profile [81].

6.3.3 Nucleic acids

Gene therapy involves intracellular delivery of genetic materials such as nucleic acids to replace, augment, or repress defective genes or biological functions as therapeutic effects. Gene therapy can be especially effective in treating diseases that are caused by mutations of a single gene defect, such as in the case with CF and α_1-antitrypsin deficiency. Other acquired respiratory disorders such as asthma, COPD, and lung cancer may also be candidates to be used for potential gene therapy since they may also be characterized by an imbalance of damaging and protective mechanisms. A primary advantage of aerosolized gene therapy would provide a direct, noninvasive delivery route to different targeted regions of the lung that can withstand the administration of optimal doses to those sites while limiting systemic side effects. Together with its potential for drug delivery, the lung is also an attractive organ for gene therapy since it can be accessed by both airways and

vasculature. When the airways are used as a platform for gene transfer, the gene expression mainly takes place in lung epithelial cells with the compartmentalization of the transgene within the lung and there is little systemic distribution [163].

However, delivery of nucleic acids such as DNA for gene therapy by inhalation is still in its infancy. Polymeric nanoparticles, such as those prepared with gelatin and PLGA, are considered to be promising carriers of DNA to the lung due to their biocompatibility, ease of surface modification, localized action, and reduced systemic toxicity [90].

The discovery of RNA interference was first established in the nematode, *Caenorhabditis elegans* in the late 1990s through an active process of double-stranded RNA (dsRNA) cleavage which induces gene silencing [164]. There is another class of RNAs known as the miRNA that are endogenous noncoding RNAs ~22 nucleotide long that control the gene expression of target genes at the posttranscriptional level [165, 166].

In pulmonary drug delivery, RNA delivery has been explored by several researchers to explore formulation strategies and to study their utility in gene therapy. Spray-drying has been the preferred technique to prepare powder formulation of small interfering RNA (siRNA), and siRNA-containing PLGA nanoparticles intended for inhalation have been reported to have low water content and aerodynamic particle size suitable for inhalation [167, 168]. Among different types of RNA, siRNA has been well explored for inhaled delivery of nanoparticle formulations with several nanoparticles and nanogels of siRNA being shown to be effective against pulmonary inflammation and lung tumors [169–171]. Once a siRNA has been designed, a potential delivery system is needed. Viral vectors have been developed for efficient delivery to a range of mammalian cells, where the genetic material is placed within the vector. Retroviral vectors have been designed to allow for efficient, uniformed delivery, and effective knockdown in cells. However, viral vectors have many disadvantages, as mentioned previously. One primary disadvantage is the induction of an immune response to the virus that can impede gene delivery and result in severe complications for a patient. Nonviral vectors such as lipid or polymer-based nanoparticles have gained interest as carriers for siRNA, with promising results. With siRNA and DNA being negatively charged along with the cell surface, the ability for positively charged lipids or polymers can aid in the transfection as well as the packaging of these genetic therapies. With the production of particle engineered siRNA carriers the addition of targeted moieties can be applied to achieve the transfection of a particular cell type [172–175].

Similarly, miRNAs have also been investigated for their inhaled delivery using nanoparticle formulations. Mohamed et al. formulated the nanocomposite microparticles of miRNA-146a-loaded PGA-co-PDL nanoparticles for pulmonary delivery to treat COPD [134]. McKiernan et al. successfully investigated the targeting of miRNA-126 loaded in polymeric nanoparticles, polyethylenimine (PEI), and chitosan in airway epithelial cells in the treatment of CF. The study found that PEI-loaded nanoparticles were more effective than chitosan-loaded nanoparticles; whereby a significant knockdown of TOM1 expression was observed [176].

6.3.4 Vaccines

Nanoparticle vaccines for inhalation are an emerging technology for the development of effective vaccines for several current and new respiratory infectious disorders and pulmonary immunization can be more effective over parenteral vaccine in such infections since improved immune responses can be achieved by pulmonary immunization [177, 178]. The rationale for aerosolizing vaccines is based on three advantages: (1) avoids the need for consistent strategies in the disposal of the mass number of needles that will be required for vaccination campaigns in developing countries while limiting the transmission of blood- borne pathogens by improper use, handling, and disposal of sharps; (2) induces protection by the airway mucosa to establish a first line defense against pathogens that normally enter the body by the mucosal surfaces; (3) and finally, there is a potential for better immunization responses in children whose maternal antibodies tend to interfere with subcutaneous immunization while appear to have little to no interference in mucosal immunization [173, 179, 180].

The development of nanoparticle systems for vaccine delivery poses several advantages including similar size to pathogens allowing the immune system to respond allowing for antigen release and exposure. Preparation of vaccine formulations for inhalation is relatively simple and can be prepared using techniques such as spray-drying and spray freeze drying [178], and inhalable dry powder vaccine formulations have been successfully formulated and tested in animals as well as humans (e.g., measles dry powder vaccine for inhalation) [181, 182]. For polymeric nanoparticle formulations of inhaled vaccines, naturally occurring polymers such as chitosan, are gaining more attention in recent years. Additionally, many inorganic nanoparticles can be used as tools in biomedical applications. Inorganic materials such as iron oxide, quantum dots, carbon, silica, gold, and silver are being used as antigen and adjuvants delivery carriers. Particle size is one of the crucial physicochemical parameters which affects the degree of phagocytosis by macrophage and activation of dendritic cells in vitro. For improved immunogenicity and antigen presentation, inorganic nanoparticles can be conjugated with a wide range of molecules, including adjuvants and antigens at high density. The utmost common nanoparticle used for vaccination is gold owing to its numerous advantages such as biocompatibility, tunable size and shape, simple conjugation steps for antigens and adjuvants, self-adjuvant effect, and traceability by imaging. The efficient delivery of vaccines, loaded in nanoparticles, can then be effectively formulated to be delivered by aerosolization. The delivery by the inhalation route of such vaccine formulations is promising for improved immunization from the pulmonary route.

6.4 Devices for inhaled delivery of nanomedicines

Different types of inhaler devices can be employed for inhaled delivery of nanomedicines to aerosolize them based on their formulation types such as dry powder inhalers and

nebulizers for powder and liquid forms respectively. The development of an appropriate inhaler device according to the particle type and the loaded drug is equally essential together with the development of nanoparticle-based formulation. Since the factors such as the type of the device used, the flow rate, and the volume administered also affect the bioavailability and the therapeutic effect of the formulation, it is important to assess the formulation together with its intended delivery device [108]. The devices that can be used for precise inhaled delivery of nanomedicine include the dry powder inhalers, the pressurized metered-dose inhalers and the nebulizers including air-jet, ultrasonic, and vibrating-mesh nebulizers [183].

7. Toxicity of inhaled nanomedicine

Nanoparticle-based drug delivery systems provide promising opportunities in the treatment of lung diseases. However, the number of studies on safety and tolerability of pulmonary delivered nanoparticles is relatively low.

Effects of nanoparticle surface charge have been studied for polymeric particles [184]. The differential pulmonary toxicity of positively and negatively charged PEG-Polylactic acid (PLA) nanoparticles was investigated following daily endotracheal instillation to BALB/c mice. Cationic stearylamine-based PEG-PLA nanoparticles elicited increased local and systemic toxic effects. In contrast, anionic nanoparticles of similar size were much better tolerated but still showed local inflammatory effects. No pathological observations were detected in the internal organs following instillation of anionic nanoparticles.

It has been found that nanoparticles induce their toxicity by increasing intracellular reactive oxygen species (ROS) levels leading to oxidative stress and increase in the levels of pro-inflammatory mediators, which in turn causes DNA damage and cell death [185]. Toxicity of inhaled nanoparticles is also contributed by the excipients, mostly composed of polymers, used in the formulation to form the nanoparticle matrix. Many nanoparticles either organic or inorganic as well as nonbiodegradable polymeric nanoparticles are reported to induce inflammation in the lungs and are not safe for pulmonary drug delivery [186]. This has led to a growing interest of researchers on biodegradable nanoparticles which are promising vehicles for pulmonary delivery of drugs. Biodegradable nanoparticles have shown promise in being safer to the lungs compared to the nonbiodegradable materials [186, 187]. Nevertheless, tolerability and safety of nanoparticles after inhalation is still a concern from the regulatory perspective. Although nanoparticles smaller than 200 nm in size can escape detection by alveolar macrophages [90] and avoiding the immune system may be useful in terms of drug delivery and delivery efficacy, nanoparticles, on the other hand, may pose a risk of toxicity due to their continued retention within the lungs or the biological system. Moreover, particles in the nanometer range that reach the alveoli are likely to cause an inflammatory reaction different to that from

the larger particles [188]. In respiratory disease, this type of pro-inflammatory reaction is highly unfavorable and delivery of toxic nanoparticles to the lungs can have adverse effects on the surrounding cells and tissues.

8. Future perspective of pulmonary delivery of nanomedicines

With ever-growing interest in the use of nanotechnology to improve day-to-day life, new and overlooked areas need to be explored. As this technology progresses and new nano-sized formulations begin to expand, toxicity and safety will need to be looked at more in-depth, since there is a lack of information available.

The delivery of drugs as well as gene therapy to the lungs by aerosol route can be enhanced with the use of nanoparticles. These particle systems can provide controlled and targeted delivery once administered to the site of infection within the lung. Further studies in efficacy and performance clinically will need to be explored.

There are much hope and promise for the field of nanotechnology within the pulmonary route. Not only can lung associated diseases and immunization be modified by nanocarrier systems, but systemic delivery may be achieved by the use of nano-sized particles that may readily transport through the alveolar regions of the lung.

The effect of residence time and chronic exposure of nanoparticles to the lung will need to be looked at further. With studies in environmental fine particles indicating potential pulmonary adverse effects after chronic exposure or prolonged time in the lung, these concerns will need to be shown to be specific for environmental particulates and not applicable to nanoparticle drug delivery systems for extensive research to continue.

With ever-growing interest and understanding in the effect of physical and physiological parameters needed for total and regional deposition of aerosol particles, the influence on particle development has progressed. A variation on these parameters is being used to optimize deposition and overall drug payload to sites of interest. Nanoparticle delivery systems may provide unique opportunities to solve difficult pulmonary drug delivery problems.

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CHAPTER 15

Nanomedicine in pain management

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1. Introduction

According to International Association for the Study of Pain (IASP), the definition of pain is "an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage" [1]. It involves a complex process of neuronal signaling in the central nervous system and peripheral nervous system. The definition of pain entails that pain is a personal experience and every person elucidate it according to their prior life experiences with an injury. And the perception in pain varies from individual to individual. Many factors play an important role in the perception of pain and also in response to pain like cultural factors, gender, genetic, and environmental factors. The strong association of these social and psychological factors which are not so easy in clinical practice and sometimes hinders the correct diagnosis and accurate treatment is hindered due to the personal perception for pain and it makes pain as a massive public health problem [2]. Pain aids as a protective role in the alertness of a person in case of tissue injury as well as threats to bodily integrity and survival. Moreover, deprivation of the existence of pain as a priority health condition from so many years has caused less understanding of the mechanism and pain management as well as affecting the development along with the execution of advanced therapies. Nowadays, progress is underway to modulate the classical view of pain and aiming for its recognition as a genuine health problem or better as a unique medical disease rather than a symptom of a disease [3].

From the clinical point of view, the classification system of pain is useful for evaluation of pain or diagnosis and also for proper treatment of the pain. Pain is classified according to specific characteristics, i.e., based on its anatomical location, duration, severity, and its etiology as discussed in Fig. 1. Types of pain are as follows:

(1) Based on anatomic location (Bones, Tendons, Joints, Viscera, and Muscles)
(2) Based on pain physiology (Inflammatory, Neuropathic, and Nociceptive)
(3) Based on pain duration (Chronic, Acute, and Breakthrough)
(4) Based on intensity (Mild, Moderate, and Severe)
(5) Based on etiology (Malignant and Nonmalignant)

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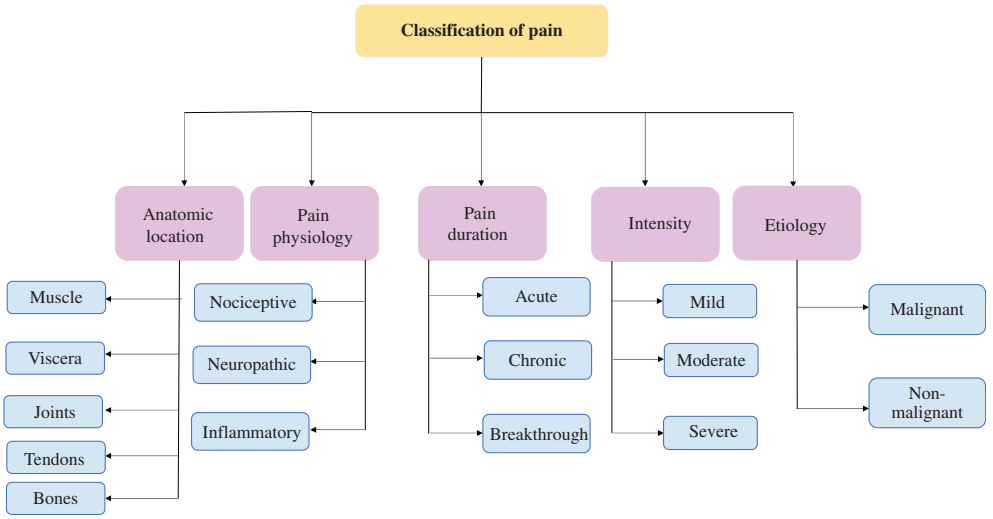

Fig. 1 Classification of pain.

Clinically for the description of pain, mostly pathophysiological classification is used and different types of treatment are used for a different type of pain. Nociceptive, inflammatory, and neuropathic pain have different clinical approaches for treatment. In induction of nociceptive pain, specific pain receptors, i.e., nociceptors are stimulated which are very sensitive to stimulus. In this case, pain works as a primary warning system for physiologically protective and to circumvent contact with any of the harmful stimulus [4]. Nociceptive pain is also further classified as somatic or visceral pain according to the location of the pain. The injury occurs into superficial and deep tissues and results in somatic nociceptive pain. Whereas, the visceral nociceptive pain induces the nociceptors stimulation present in the visceral organs or internal organs and it is classically diffused, difficult to locate, generally constant, and occasionally allocated to a far body part. Examples of somatic nociceptive pain are musculoskeletal (myofascial pain, joint pain), cutaneous and examples of visceral nociceptive pain are smooth muscle and hollow organs. In somatic nociception, five physiological processes are involved [5] which are discussed below.

1. Transduction: Any chemical, mechanical, and thermal noxious stimuli act via peripheral nociceptors with conversion into electrical activity, later, terminating in an action potential which supports nerve impulse.
2. Conduction: The first-order neurons, nerve impulse travels and reach the second-order neuron to form synapse.
3. Transmission: At the synapse, synaptic transmission of information takes place between both first-order and second-order neurons present in the dorsal horn basically in the spinal cord.

4. Perception: Experience of pain actually with sensory as well as effective aspect.
5. Modulation: Experiencing pain is not direct but noxious stimuli arise due to proportionate mechanical response with an assemblage of factors modulating the stimulus-response pathway.

In the healing process associated with the damaged region after the inevitable injury to the tissues or nerves, the immune system is activated and triggers inflammatory response. Reason for activation of the immune system and inflammatory response is for the eradication of potential pathogens, clearance of injured tissues, rebuilding the homoeostasis, and the anatomical and physiological reestablishment of the affected area. Additionally, as a result of this inflammatory response as well as provisional sensitization due to nociceptive system, a condition in which generation of pain hypersensitivity or tenderness in the injured area and local non-swollen tissues, arrests further damages. Hence, the atypical sensory processing infers that any inoffensive stimuli that would usually not produce pain and noxious stimuli might cause an amplified pain response. Pain that arises due to this is known as inflammatory pain which is protective, valuable, and adaptive for a successful regaining. Normally, this pain vanishes along when the injury state is resolved. Though, under certain pathophysiological or physiological conditions, nociceptive signaling can function abnormally that results in pain with no obvious defensive or reparative role. In above-discussed cases, pain is determined which might last as long as inflammation resides [6]. Examples of inflammatory pain are rheumatoid arthritis, appendicitis, herpes zoster, inflammatory bowel disease, and many others.

Neuropathic pain is a nonadaptive and multifactorial pain which is caused by a lesion or may be due to dysfunction in the nervous system. Neuropathic pain is redefined by some expert, that pain arises due to lesion or disease of the somatosensory system [7]. Neuropathic pain is subcategorized into central and peripheral neuropathic pain. Neuropathic pain is frequently related to the advent of symptoms, for example, hyperalgesia, allodynia, dysesthesia, and lancinating pain. Examples associated with neuropathic pain are diabetic neuropathy, postherpetic neuralgia, phantom limb (postamputation) pain, poststroke central pain, and spinal cord injury pain. This chapter is designed to focus on the concepts in context to pain and nanomedicine development for counteracting the pain.

2. Pathophysiology of pain

2.1 Pathways for pain

Ascending pathway: Three types of neuron-based pathways.

I. First-order neuron: These neurons start from the periphery (bone, skin, muscles, ligaments, and other viscera) traveling via the peripheral nerve and reaches the spinal cord, i.e., to the dorsal horn.

II. Second-order neuron: It starts from the dorsal horn and crosses over to the contralateral side with a further ascent in thalamus via the spinal cord, and other brain areas like dorsolateral pons.
III. Third-order neuron: Also known as a tertiary neuron. It starts from the thalamus with termination in the cerebral cortex.

Descending pathway: This pathway starts with the limbic system of brain, parabrachial area, rostral ventromedial medulla, and periaqueductal gray nucleus raphe magnus.

2.2 Nociceptors

Primary afferent neurons or first-order neurons transfer nociceptive information through peripheries into the spinal cord, and it is conveyed to the thalamus and cortex. For the generation of action potentials, ion channels are crucial. In our primary afferent system, nociceptive neurons are an important part and it conveys information from the periphery towards the CNS. These are also called first-order neurons because these are the first neurons in conveying signals to the CNS. They have high activation threshold that is essential for the initiation of depolarization and by their non-capsulated, free nerve endings, differentiated from other sensory neurons. Primary afferent neurons are having specialized receptor organs like muscle spindles, but they are absent in primary afferent nociceptors. Most of the bodily anatomical structures include musculoskeletal tissues, skin, and the viscera fine nerve endings of primary afferent nociceptors. Primary afferent nociceptors are not innervated in the brain so the damage to the parenchyma creates an inflammatory response but it does not create any feeling of pain. That is why the brain surgeries are possible without the use of analgesic agents in brain tissues in a conscious patient. Each primary afferent nociceptor comprises its receptor region, i.e., fine nerve endings, an axon that projected between the target structure along with the spinal cord, a cell body placed within the central processes that project to lamina I and II of the dorsal horn and dorsal root ganglion of the spinal dorsal root. Presynaptic terminal is formed by these processes which are excitatory in nature and release of glutamate onto postsynaptic neurons. Transmission way of nociceptive information starts from the body to the brain stem, in the anterolateral system/spinothalamic tract via the spinal cord and the ventral posterolateral nucleus of the thalamus. Nociceptive-based signals can directly impact physiological function at the brain stem and the thalamus conveys information of tissue damage to numerous cortical tissues. Pain is not perceived until this point of the pathway. The head portion of the trigeminal nerve is anatomically analogous to the somatosensory nerves. Within the trigeminal ganglion presence of cell body followed by central projections with termination on postsynaptic neurons arising to the thalamus in the spinal trigeminal tract of the medulla [8].

In order to transmit information with damaging of tissue to higher-order neurons, primary afferent nociceptors must translate a thermal, mechanical, or chemical stimulus with an action potential and afterward releasing chemical neurotransmitter from dorsal

horn. Sensory transduction region of the nociceptor is provided by free nerve endings and activating ion channels stimulus is converted into a receptor membrane potential. In maintenance and fluctuation of charge across the receptor membrane Na^+, K^+, and Ca^{2+} are involved. Depolarizing the sensory area of the primary afferent nociceptor membrane is led by the opening of these cation channels, will be referred to as the generating "receptor potential." It is similar to the "action potential" created along the axon. After reaching threshold and resultant receptor potential transforming into a series of action potentials with repetition of propagation with transmission to synaptic signaling to terminals along the full length of axon. Voltage-gated Na^+, as well as K^+ channels, generate depolarization of axon. Release of neurotransmitter must occur at the dorsal horn to send signaling via postsynaptic afferent neurons and it is mainly mediated via voltage-gated Ca^{2+} channels. Many factors modulate any stage in the process [9].

2.3 Categories of primary afferent nerve fibers

According to the conduction velocities afferent nerves are classified. Morphology of these axon governs the conduction velocity. Conduction is faster with the large diameter of neuronal axons or due to myelination. Conventionally, it was supposed only Aδ and C fibers with slowest conducting velocity were supported by signaling through noxious stimuli and can be perceived as pain. Though, progressively it is understood that there is an overlapping of greater functional present between fiber groups. For conduction of signals from noxious stimuli, Aβ fibers may also be responsible, whereas Aδ and C fibers can conduct signals from the non-noxious thermal and mechanical stimulus. Fig. 2 represents the various types of primary afferent nerve fibers. According to their

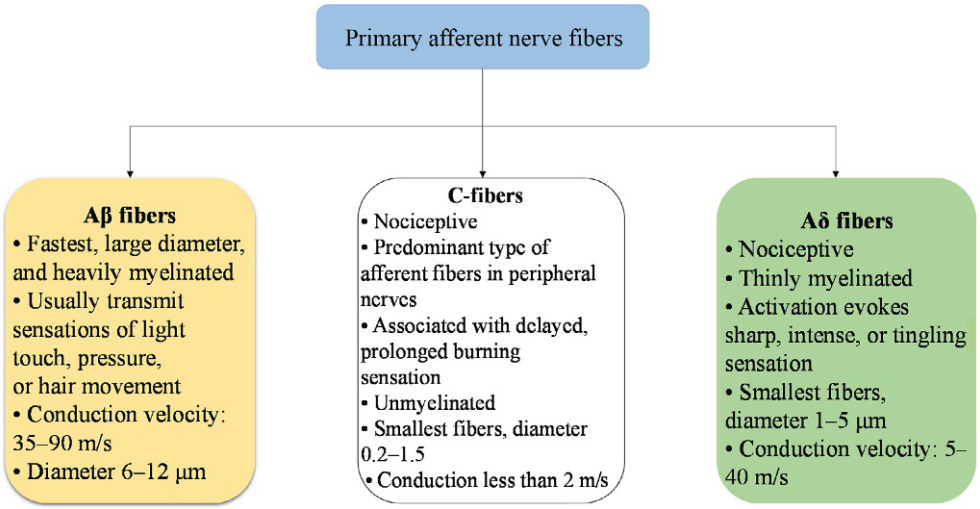

Fig. 2 Types of primary afferent nerve fibers.

location nociceptor threshold also differ. Cutaneous primary afferent nociceptor and those supplying muscle are differentiated from the low threshold large-caliber non-nociceptive afferents because former have a high threshold of activation [8, 10, 11].

2.3.1 Peripheral pathway in pain

Transduction at free nerve ending is started by nociception and somato-sensation associated noxious stimuli. Further, it activates the peripheral primary afferent fibers. Cell bodies of these primary afferent fibers are present in the dorsal root ganglia. Aδ-fibers have faster conduction velocity than C fibers and transmit the first pain sensation as quick prickling pain is felt. Suspended burning sensation or aching is caused by the transmission of second pain sensation by slower C fibers. Before the propagation along with the Aδ- and C-fibers, noxious stimulus is changed into chemical and electrical signals.

Signals have to be encoded appropriately for the efficient travel of noxious stimulus from the periphery to brain. Transduction function is carried out by the free nerve endings of the primary afferent fibers and to modulate pain transduction many factors are involved. Mainly, the chemical atmosphere or microenvironment affects the pain transduction. Many potent modulators contribute to the inflammation in tissue engineering. Some factors have dual role in the initiation of the inflammatory response to tissue injury and in modulation of the pain, for example, bradykinin, serotonin, and histamine [12]. In potentiating pain, some cell-derived factors act directly on nociceptors, for example eicosanoids (arachidonic acid derivatives, prostaglandins, thromboxane, and leukotrienes) [13]. Cytokines like tumor necrosis factor α also contribute to pain modulation by sensitizing nociceptors. These factors are balanced by compensatory mechanisms and pain transduction thus the nociceptor sensitization is lessened. In the setting of inflammation, opioid receptors are upregulated by afferent fibers. To counter extreme cytokine activity somatostatin is helpful. To desensitize C fibers, acetylcholine working at muscarinic receptors also work [10].

2.3.2 Pathway of spinal cord: The dorsal horn

In a body where primary afferent fibers originate, primary afferent fibers which convey painful or nonpainful stimulus terminates in the spinal horn. In laminae layers, the dorsal horn is anatomically ordered which is known as Rexed laminae [14]. Different afferent fibers terminate in different laminae. A prominent example is of nonpain larger Aβ-fibers termination in laminae III-V, Aδ-fibers termination in laminae I and III-V, and C-fibers termination mainly in the most superficial laminae (I and II). Nociceptive-specific (NS) neurons and wide-dynamic-range neurons (WDR) are involved in spinal trigeminal projection neurons and second-order nociceptive spinal. Aβ, Aδ, and C-fibers send somatosensory inputs to the wide dynamic range cells which are intense in deeper laminae (III-IV) and both noxious and innocuous stimulus activate them. With lowering in the stimulation of Aβ-fibers from mild nonpainful sensation to intensifying pain at higher levels of stimulation, a graded response is produced by wide dynamic range cells.

In superficial laminae, I and II lie nociceptive-specific neurons which do not show graded response or activation. When noxious stimulus surpasses a high threshold then nociceptive-specific cells fire. Midline proximal to the spinal level is crossed by the axons of wide dynamic range along with nociceptive-specific second-order neurons. In the contralateral, anterolateral quadrant of the spinal cord, they gather in the form of bundles and then target towards in the brainstem, diencephalon, cortex, and rise in the spinothalamic tract (STT). Neurotransmitters released from the spinal cord modulate the noxious input only at the spinal level. Development as well as maintaining chronic pain states involves spinal modulation of a special type, i.e., central sensitization [15]. For a given stimulus, firing frequency as well as the intensity of spinal neurons is increased by threshold noxious stimuli and leads to central sensitization. The dorsal horn spinal transmission cells, persistent nociceptive signaling from C-fibers cause wind up phenomena. Central sensitization is triggered by the barrage of C-fiber transmission to wide dynamic range cord cells. Increased wide dynamic range firing, allodynia, and hyperalgesia characterized this pathway [10].

2.4 Ascending pathway of pain transmission

For conveying noxious or painful information to the central system, the spinothalamic tract is the chief ascending pathway in the spinal cord. In the anterolateral part of the spinal cord, the spinothalamic tract is present. Lateral pathway and the medial pathway are the two components of the spinothalamic tract. The nature and physical location of the noxious stimulus, i.e., the sensory discriminative feature of pain perception is determined by the lateral pathway. No spatial information or attributes of the noxious stimuli is carried by the medial pathway. Motivational-affective component of pain perception is encoded by the medial pathway, i.e., to focus on and contain the pain, conscious emotional urgency is created by the medial pathway [16].

In the anterolateral quadrant of the spinal cord, with spinothalamic tract nonpainful sensory information transmittance, the dorsomedial nucleus gracilis is activated eventually after the transmission of the nonpainful stimuli through the dorsal columns. The dorsal horn, at the level of first-order synapse sides are not crossed by the dorsal column pathway, as opposed to the spinothalamic tract. Alternatively, nonpainful sensory input is carried by dorsal columns up to the brainstem, nucleus gracilis, and nucleus cuneatus, both form medial lemniscus by crossing the midline. The spinothalamic tract rises to the brainstem, the diencephalon and numerous cortical sites which are tangled with somatosensory processing at the supraspinal level [17]. Spinal projection neurons are activated by fibers from the spinothalamic tract parallel pathways in the anterolateral cord and then terminated into brainstem and midbrain nuclei. Those sites are included in the brain stem which controls autonomic functions such as rate of respiration, pulse, and blood pressure observed in persons in pain. Descending and ascending modulation of painful and nonpainful transmission is affected by the sites involved in the midbrain. Fear and emotional

arousal related to pain is contributed by the amygdaloid nucleus in the temporal lobes which is the projection of the midbrain parabrachial nucleus. Pain-related neuroendocrine changes leading by hypothalamic activation, such as the release of corticotropin-releasing hormone (CRH) increases and elevates the blood level of cortisol. Fig. 3 represents schematic representation describing the pathophysiology of pain.

Spinothalamic tract fibers terminate at the ventral posterior medial nucleus and ventral posterior lateral nucleus at the level of the thalamus. From the body surface, inputs are received by the ventral posterior lateral nucleus and from the face, inputs are received by the ventral posterior medial nucleus. A third-order projections which characteristically are diffused, projects to the lobe, neocortex, and prefrontal cortex. From the core of the ventral posterior lateral nucleus, neurons projects to somatosensory cortical area SI as well as SII along with the insular cortex. Medial thalamic nuclei forecasts to the anterior cingulate cortex. In varied series of pain states in human, activation of cortical areas, anterior cingulate cortex, primary as well as secondary somatosensory cortices, insular cortex, and medial prefrontal cortex is shown in Rapps et al. [18]. In diverse aspects of pain, these areas play an important role, e.g., emotional dimension of pain is contributed by cingulate cortex and medial prefrontal cortex's activation and to help in focusing cognitive-analytic processes on pain, the insular cortex may work [19]. Robust and reciprocal interconnection of brain regions occurs at the same time. Effective reactions, pain intensity, and cognitive assessment of the seriousness of pain are integrated and controlled by both [20]. During pain, the interconnections and clear serial stimulation of these brain regions are exposed by the various dynamic brain imaging techniques, which includes magnetoencephalography, positron emission tomography (PET), and

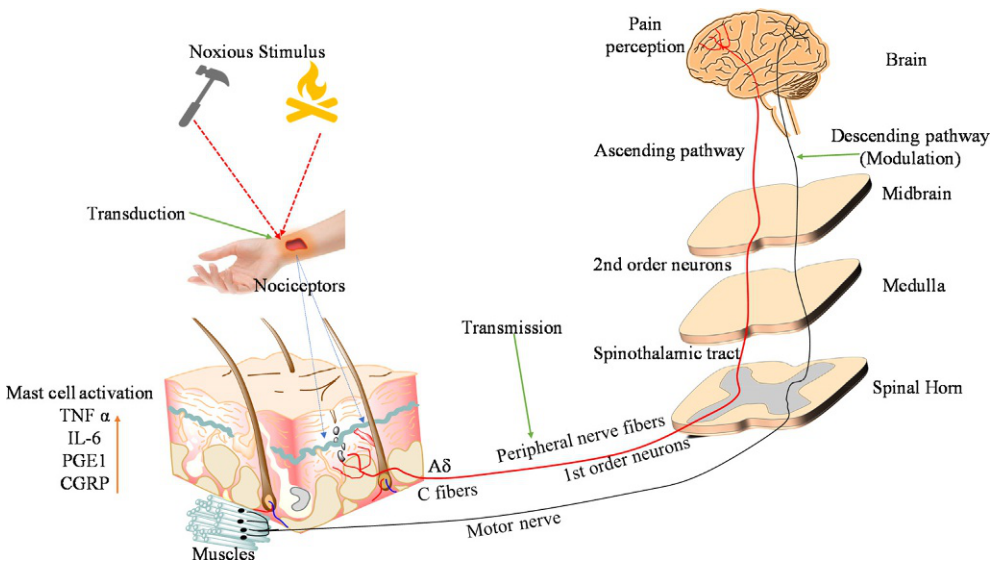

Fig. 3 Schematic representation describing pathophysiology of pain.

functional magnetic resonance imaging (fMRI) [21]. These areas are considered as most eminent components of the pain matrix of the brain. Connectivity and stimulation of multiple cortical and subcortical structures affect the human consciousness, it is not related to a single brain region [10].

2.5 Descending modulation of pain

Dorsal horn shows both inhibitory and facilitatory effects on the primary afferent neurons by the supraspinal descending modulation of nociception. In pain transmission, from the devaluation of the acute pain to the expansion of chronic pain, descending modulation has diverse effects. In descending modulation, well-characterized supraspinal components are periaqueductal gray and rostral ventromedial medullary pathways. Cortical and limbic region of the pain matrix of the brain sends descending projections to them. In the dorsal horn, spinal nociceptive-specific and wide dynamic range neurons receive either inhibitory or excitatory impulses which are projected by the periaqueductal gray to the rostral ventromedial medullary. In the dorsal horn, inhibitory and excitatory effects are facilitated by the rostral ventromedial medullary via ON and OFF cells [22]. For descending inhibition OFF cells are essential and except during nociceptive input they are tonically active and analgesics like morphine activates it. Increased pain signaling tends by active ON cells which became active during nociceptive inputs. Chronic pain stress implicates inhibition of OFF cells and activation of ON cells.

In descending modulation, several neurotransmitters are involved. In the spinal cord, the predominant inhibitory neurotransmitter is glycine, whereas levels of GABA predominates at the higher central nervous system. Sometimes different effects are shown by the same neurotransmitter in the periphery and central pathways. For example, central pain is suppressed through descending pathways and at the site of trauma and transduction GABA can show inhibitory or facilitating effects. In the spinal cord, interneurons are stimulated by norepinephrine through the dorsolateral funiculus to reduce pain transmission [23]. Vital therapeutic tools for abnormal peripheral afferent activation-related conditions like trigeminal neuralgia, peripheral neuropathies, and post therapeutic neuralgia are the drugs that hinder the reuptake of serotonin and norepinephrine [10].

3. Nanomedicines and pain

There are many organic and inorganic nanoparticle-based formulation which is used to deliver many drugs like NSAIDs, anesthetics, opioids. To improve the quality of patient's life and to increase the safety and efficacy of the drug nanomedicines are formulated. Nanomedicine-based technologies can also become useful in the diagnosis of the disease. For the repairing or healing of the damaged nerve tissue, spinal cord injuries, and other types of injuries like bone and cartilage which are related to extreme pain, advancements in tissue engineering is very useful. Various types of nanomedicines to increase the advantages or to decrease the limitations of present formulations are given in Table 1.

Table 1 Nanomedicines used in pain management.

Drug delivery system	Drug	Route of administration	Pharmaceutical advantages	References
Solid lipid nanoparticles (SLNs)	Lidocaine	Epidural	SLNs extended and controlling Lidocaine action in epidural anesthesia	[24]
	Lidocaine	Topical	Provides long duration of anesthesia	[25]
	Indomethacin	Ocular	Surface modification with chitosan chloride increased permeation	[26]
	Piroxicam	Topical	Enable sustained release	[27]
	Diflunisal		Skin retention and better permeation and also escalate therapeutic efficacy	[28]
	Celecoxib	Intra-articular	Prolonged anti-arthritic activity of celecoxib	[29]
	Morphine	Topical	Prolonged morphine release	[30]
Nanostructured lipid carrier (NLCs)	Benzocaine and lidocaine		Prolonging anesthetic effect of Benzocaine and Lidocaine	[31]
	Lidocaine and prilocaine		Sustained release effect	[32]
	Oxaprozin	Oral	Highly potential in comparison to oral administration and significantly reduced gastric side effects	[33]
	Flurbiprofen	Ocular	Sustained release using NLC and no toxicity	[34]
	Indomethacin		Increase drug loading as well as entrapment followed by delivery to anterior and posterior segment ocular tissues	[26]
	Lornoxicam	Topical	Increased the skin penetration rate of the drug	[35]
	Ibuprofen		Increase the bioavailability and the therapeutic efficacy	[36]
	Celecoxib		Faster onset as well as prolonged activity	[37]
	Meloxicam		Sustained release followed by enhanced the skin permeation	[38]
	Indomethacin		Delayed and sustained anti-inflammatory effect	[39]

	Ketoprofen and naproxen		Prolonged effect	[40]
	Valdecoxib		Faster onset and prolonged effect	[41]
Adamantane-based dendron	Ibuprofen	Transdermal	Increase in anti-inflammatory activity	[42]
Polyamidoamine dendrimer	Indomethacin		Enhancing flux across the skin	[43]
	Ketoprofen		Enhances solubility of Ketoprofen	[44]
Cationic carbosilane dendrimer	Ibuprofen		Higher anti-inflammatory properties	[45]
Triazine-based dendrimer	Ketoprofen		Enhances solubility of Ketoprofen	[46]
Liposomes	Prilocaine		Prolonged the duration of anesthesia	[47]
	Lidocaine		Improvement in procedural success rate, reduces procedure time and reduction in pain	[48]
	Mepivacaine	Intraoral	Increase in the anesthesia duration as well as injection discomfort	[49]
	Ibuprofen	Epidural	Enhances dural permeation	[50]
	Diclofenac	Oral	Improved drug delivery and efficacy	[51]
	Diclofenac		Act as a sustained release depot	[52]
	Meloxicam	Transdermal	Menthosome and transferosome showed higher flux	[53]
	Hydromorphone	Subcutaneous	Prolonged relief from the neuropathic pain	[54]
	Fentanyl	Intranasal	Long-lasting analgesic effect	[55]
PCL nanospheres	Lidocaine	Infiltration	Reduction in toxicity as well as prolonging the action	[56]
PLGA nanoparticles	Bupivacaine	Infiltration (subplantar region)	Sustained analgesic and anti-allodynic effect	[57]
PLGA nano-capsules	Benzocaine		Improvement on intensity and duration of analgesia	[58]
	Bupivacaine		Good physicochemical stability	[59]
PLGA Nanospheres	Ropivacaine		Decrease the systemic toxicity	[60]
PLA or PCL nano-capsules	Benzocaine		Sustained release and enhanced anesthetic effect	[61]

Continued

Table 1 Nanomedicines used in pain management—cont'd

Drug delivery system	Drug	Route of administration	Pharmaceutical advantages	References
Eudragit RL and PLGA NPs	Piroxicam	Intra-articular	Diminish systemic exposure and extend retention time of Piroxicam in the joint	[62]
Eudragit S100 Nanoparticles	Ibuprofen	Oral	Sustained drug release and good stability	[63]
PCL Nanoparticles	Meloxicam		Improved the anti-inflammatory activity of Meloxicam	[64]
Eudragit L 100 nano-capsules	Indomethacin		No genotoxicity of nano-capsules in leukocytes and HepG2 cells	[65]
Ethyl cellulose nanoparticles	Piroxicam		Ethyl cellulose-NPs have reduced ulcerogenicity	[66]
Polypyyrole nanoparticles	Piroxicam		Sustained release of Piroxicam	[67]
PLGA nanoparticles surface modified with octa-arginine	Loperamide	Intranasal and intravenous	Sustained drug release and targets to CNS	[68]
PNIPAM nanogel	Bupivacaine	Peritoneal	At higher concentration well tolerated in in vivo with minimal cytotoxicity	[69]
Chitosan thermogel	Ropivacaine and Dexamethasone	Transdermal	Controlled-release system	[70]
Ethosomes	Diclofenac sodium		Better permeation through rat skin	[71]
	Ketoprofen		Higher transdermal flux	[72]
	Lidocaine		Improvement of stability, high entrapment efficiency and enhancement in permeation	[73]
	Ropivacaine		Increased the transdermal flux and permeation	[74]
	Benzocaine		Improved intensity and duration of anesthetic effect	[75]
	Ibuprofen		Safe and does not cause irritation	[76]
	Piroxicam		Rapid onset and maximum release of drug with reduction of side effects	[77]
	Acelofenac		In vivo efficiency of Acelofenac increased	[78]
	Meloxicam		Rat paw edema readily reduced	[79]

Micellar nanogel	Lidocaine and prilocaine	Topical	Enhanced intensity of anesthetic effect	[80]
Polyethylene oxide and polypropylene oxide block copolymer micelle	Lidocaine and prilocaine		Enhances release and stability	[81]
Micellar solutions using single and mixed surfactant systems	Naproxen		Targeted profiling, with enhancement in bioavailability and controlled rate of release	[82]
Complex micelle	Ibuprofen		Avoidance of burst release and sustained release profile was observed	[83]
Mesoporous silica nanoparticles (MCM-41)	Ibuprofen	Subcutaneous	Improve the bioavailability of the drug and but no enhancement in COX-1 inhibitory activity	[84]
Poloxamer 407/188 binary thermosensitive hydrogels	Ropivacaine	Infiltration	Modulation of release profile with prolongation in analgesic activity	[85]
pH-Sensitive niosomes using polysorbate-20 or polysorbate-20 derivatized by glycine	Ibuprofen and lidocaine	Subcutaneous	Stable as well as non-toxic	[86]
Niosomes	Acelofenac	Topical	Niosomes showed better stability than liposomes.	[87]
	Diclofenac Sodium		Controlled and improved permeation of niosomes as compared to free dug	[88]
Niosomal gel	Lornoxicam		Enhanced skin permeation and anti-inflammatory effect	[89]
	Meloxicam	Transdermal	Superior drug release and permeation is independent to the viscosity	[90]
		Lornoxicam	Improved skin permeation and enhanced anti-inflammatory effect	[91]

3.1 Polymeric nanoparticles

Natural, semisynthetic, and synthetic polymers are widely used to prepare the organic nanoparticles which are useful in the drug delivery for pain treatment. These polymeric nanoparticles are formulated to increase the therapeutic efficacy of the medications used for the treatment of pain. Some polymers like poly (lactic-*co*-glycolic acid) (PLGA), polylactic acid (PLA), and polycaprolactone (PCL) that are degradable in nature are used in therapeutic devices which are FDA approved [92, 93, 93a, 93b]. To decrease the limitations of local anesthetics, these therapeutic devices are developed for local anesthetics, e.g., Benzocaine, Ropivacaine, Bupivacaine, etc. To increase the physical and mechanical properties of the polymeric nanoparticles, sometimes a mixture of natural and synthetic polymers and this mixture is more biocompatible than using alone synthetic polymers. The prolonged effect, controlled release, improved therapeutics effect, decreased toxicity is observed in nanoparticles formulations [5].

3.2 Liposomes

Liposomes are spherical vesicles of a bilayer of phospholipids. These lipids are amphiphilic in nature because they have hydrophilic and hydrophobic part. Liposomes are useful in drug delivery system because they can encapsulate both hydrophilic and hydrophobic drugs. Liposomes have aqueous core which is enclosed by a bilayer of phospholipid. Liposomes provide controlled and prolonged release of the drug in the treatment of pain and liposomes are having increased therapeutic efficacy and increase the penetration of the drug through the skin as observed in various formulations as shown in Table 1. In the field of dentistry, liposomes are gaining popularity for anesthetics for increasing the bioavailability that will increase the pharmacological action and for decreasing the adverse effect and toxicity of the drug. Opioids show serious systemic side effects to decrease these side effects liposomes are alternative drug delivery system. Sometimes liposomes are also used for targeting the drug by using specific ligands which have good binding affinity towards the targeted site [5].

3.3 Ethosomes

Liposomes have difficulty in crossing the intercellular channels of the stratum corneum so it becomes challenging for formulation scientist for drug permeation or penetration as a rate-limiting factor. So, to overcome this problem ethosomes that emerged in 1990s which are unilamellar or multilamellar vesicles composed of phospholipid where ethanol is used to increase permeation enhancer. Transdermal drug delivery has so many advantages because this route of administration bypasses the hepatic first pass metabolism, it avoids gastrointestinal tract problems, and easy to use for the patient. Due to these advantages, transdermal drug delivery ethosomes are getting popularity in the research area. Structure of the stratum corneum is modified by the ethosomes, which has been observed

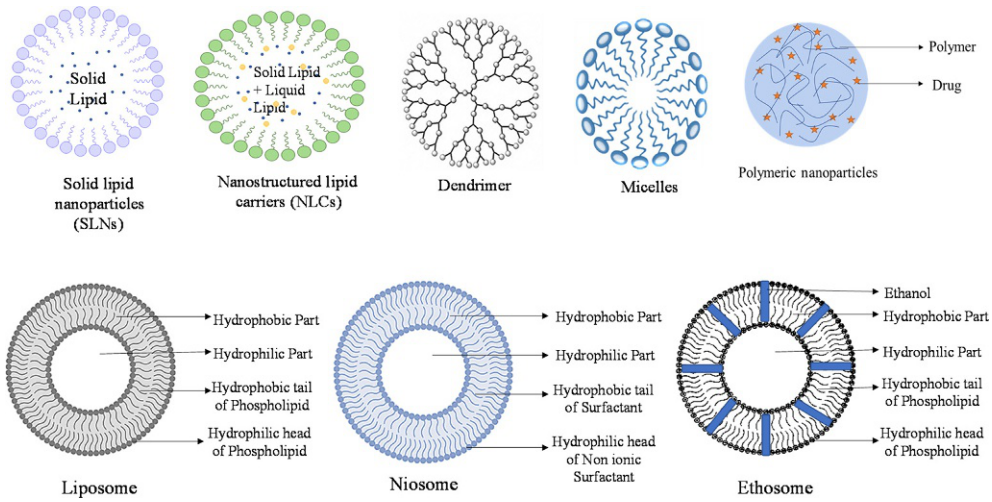

Fig. 4 Different formulations used as nanomedicines in pain management.

in some histopathological studies. Some studies prove the efficient transdermal delivery of NSAIDs and local anesthesia by the ethosomal formulations [5] (Fig. 4).

3.4 Niosomes

Nonionic surfactant and cholesterol in the aqueous phase are self-associated to form a closed bilayer structure that is known as niosomes. Niosomes are biocompatible, biodegradable, nontoxic, and non-immunogenic in nature. Like liposomes, niosomes surfactant has amphiphilic nature which possesses aqueous core surrounded by the membrane bilayer of surfactant. So, the hydrophilic drug is entrapped in the aqueous core and the hydrophobic drug is entrapped in the bilayer membrane. In topical drug delivery, niosomes are widely used due to its increased permeation through the skin. A sustained and controlled release of drug, nontoxic, and non-immunogenic nature are key characteristics of a noisome formulation. Sometimes niosomes-loaded gels are formulated so that it becomes stable and provides early ease to the patient [5]. Niosomal formulation of many anti-inflammatory drugs is formulated to increase the permeation of the drug. Examples of drugs are Rofecoxib, Lornoxicam, Aceclofenac, Nimesulide, Meloxicam, and Diclofenac diethyl amine.

3.5 Micelles

Above the level of critical micellar concentration (CMC) amphiphilic molecules get self-assembled and form nanosized core-shell structure to form the micelles. Micelles incorporation into its inner core are very useful to increase the solubility and stability

of the hydrophobic drug and due to this property micelles are gaining popularity in the drug delivery system. Due to their small size micelles are not easily recognized by the phagocytes. Micelles show long-term stability and are also helpful in controlled and targeted release of the therapeutic agent [94]. NSAIDs are perfect for incorporating within the micelles due to the side effect shown after the administration of the NSAIDs and the short plasma half-life of them. Solubilization of the therapeutic agent is increased if a mixed surfactant is used at the place of single surfactant [5].

3.6 Solid lipid nanoparticles (SLNs)

Due to some limitations of conventional carriers (liposomes, polymeric nanoparticles), e.g., low encapsulation efficiency, low stability for hydrophobic drug, solid lipid nanoparticles (SLNs) have been developed as an alternative system. SLNs are new colloidal drug carrier system which consists of lipids stabilized by surfactant. These lipids are solid both at room and body temperature. They emerged to decrease the limitations of the conventional drug carrier but they have the mixture of useful properties of conventional carriers. These solid lipid nanoparticles have many advantages, for example, less toxicity, biocompatible, biodegradable, well-tolerated, increased stability, organic solvent-free, easy scale-up techniques, and easy to sterile. Table 1 summarizes SLNs formulations with improved permeation through the skin and is also used for extended-release because it helps in prolonging the effect of drug without causing toxicity. SLNs are also used to prolong the anesthesia time of local anesthesia. Sometimes SLNs are incorporated in the hydrogels for the administration of the drug through topical route [5].

3.7 Nanostructured lipid carriers (NLCs)

Nanostructured lipid carriers (NLCs) which are known as the second generation of the lipid nanoparticles are developed to overcome the present limitations of the SLNs for example, low drug loading capacity and the drug leakage from the solid lipids during synthesis and storage. SLNs and NLCs are compared in some studies for encapsulation efficiency and in NLCs high drug loading are observed in comparison to SLNs. Many formulations are prepared as NLCs increase the permeation through the skin, increase the stability of the carrier system, and decrease the drug leakage from lipids. The drug is partitioned between the liquid lipid and solid lipid that is responsible for the sustained effect of the drug. NLCs are also formulated to increase the pharmacokinetics and pharmacodynamics properties of the NSAIDs and decrease the toxicity and adverse effects of those drugs. In-vitro studies and in preclinical studies, NLCs exhibited enhanced efficiency [5]. To improve drug delivery, NLCs of many drugs are formulated, for example, Benzocaine, Lidocaine, Prilocaine, Oxaprozin, Flurbiprofen, Indomethacin, Lornoxicam, Ibuprofen Celecoxib, Meloxicam, Ketoprofen, Naproxen, and Valdecoxib.

3.8 Dendrimers

Formulation scientist has always focused on enhancing chemical and physical properties of present conventional linear polymers. For overcoming existing nanocarriers, dendrimers are developed with peculiar molecular architecture as three-dimensional macromolecules which are extremely branched. In current years, in the field of drug delivery system, dendrimers have gained popularity because dendrimers are able to increase the solubility of the drug incorporated in it and it prevents the degradation of the drug due to the cavities present in its structure. In the dendrimer, the external surface can be functionalized with the desired therapeutic agents. Dendrimers are useful to increase the bioavailability of the drug by increasing the drug solubility and are also useful in increasing the permeation or flux of the drug through the skin. Sometimes the therapeutic agent is incorporated in the cavities also and on the external structure also and extraordinary improvement in solubility is observed [5].

3.9 Nanomedicine as diagnostic agent

Nanomaterial has the capacity to improve the sensitivity and specificity for antigen detection due to the inherent optical properties. It enables the single cell to whole-body fluid analytics as well as it is used to detect multiple analytes simultaneously because it allows for multiplexing capabilities. Nanomedicine is empowered with diagnostic applications for extraordinary development in risk mitigation strategies as well as expressing pain experience. Moreover, supplementary stress with the identification of molecular markers for neuropathic and visceral pain, nanomedicines have aided quick as well as sensitive identification of biomarkers from blood in pain conditions. This might in the future, complement the individual visual analog scale as well as other pain assessment scales which are used in the clinical setting. It appears to have substantial benefit to the effective treatment of pain if ultrasensitive nanosensors are employed for the detection of blood-borne pain related to well-established biomarkers. Specific resident blood/plasma biomarkers that are related to the pain are exposed by some investigation. Augmented plasma IL-6 has been detected in painful osteoarthritis, which includes two inflammation markers and three collagen markers. For the patient with lower back pain, potential serum biomarkers have been illuminated [95]. In patients with fibromyalgia, having raised levels of cytokine IL-6, studies have reported numerous possible blood biomarkers for pain [96, 97].

3.10 Nanochannel-based implantable drug delivery system

Potential for novel therapeutic paradigms are continuously on exploration by scientists across the world. In this 21st century, we have now aimed to work on chronotherapy and metronomic delivery which targets with more precision, on time, and its location for therapeutics with significant safety and efficacy. Delivery systems are now targeted to

release the drug at a specific target rather than to release primarily in GIT (gastrointestinal tract) and then absorption in systemic circulation to act on targeted site. Advantage of targeting at a specific site with improved therapeutic potential and minimizing side effects is further advantageous to patient in terms of compliance and an overall reduction in cost. Grattoni and coworkers have developed implant with multiple generations enabled controlled-released chips incorporated into silicone chips [98, 99]. Tailor-made surface-modified molecules are formulated and control release of the dosage form are obtained which are useful in clinical use. Nanochannels formulated technology not only provides control release by using the principle of potential difference but also a tunable style of delivery. This type of delivery system can be used for developing a smart delivery system with preprogrammed technology or remote-controlled delivery systems in comparison to hydrophilic or polymeric-based delivery systems.

3.11 Bionanoscaffolds

The era of the cold war reports the daily injury of soldiers and common people due to attacks from terrorists or military conflicts that cause trauma cases. Trauma cases cause fracture of bones and tissue damage which is very difficult to treat. After trauma from the war zone, amputation is the only option left for the surgeon. Even if amputation is avoided still it has to suffer from multiple surgeries and will take 2 years for rehabilitation along with pain which has acute and economical issues. Nanomedicine has developed a novel platform in relation to tissue engineering causing tissue regeneration by using bionanoscaffold technology. Bionanoscaffold-based technology uses a combination of existing technology. This technology focuses on expansion and isolation of mesenchymal stem cells (MSC) lines which are obtained from sheep, rat, human bone marrow, adipose tissues. After isolation of MSC, formulation of microspheres with tunable release profile [100, 101], microsphere-based delivery system is used to incorporate antibiotics, osteogenic agents, and biomolecules. Development of injectable formulation is encapsulated by incorporation of alginate microspheres. With the assemblage of these programmable-based delivery systems at the site of the fracture causes genesis of bone formation at the site of the defect.

4. Advantages of nanomedicine

1. Nanomedicine supplements the drug concentration at the site of action by active and passive targeting which results in the reduction of the drug in normal tissues.
2. Precise targeting of the drug at the site of action reduces its side effect and also improves its pharmacokinetic and pharmacodynamic profile of the drug.
3. Drug solubility is also increased by nanomedicine approach.
4. Drug stability is also increased by nanomedicine approach by decreasing its peripheral degradation in the systemic circulation.

5. Nanomedicine approach increases cellular internalization of the loaded drug by surface functionalization strategies.
6. Nanomedicines increase the time period in which the drug retains in the body so it is useful for the drugs which are removed by the body before it shows the therapeutic effect [102].

5. Hurdles in nanomedicines

In in vitro cell studies and in vivo animal studies, there is increasing postulation about the toxicity of the nanomedicines. For example, Cadmium toxicity is caused by metabolism of CdSe Qdots which will lead to an adverse effect on the viability, function, and morphologic features of primary rat hepatocytes [103]. Asbestos like inflammation and granulation in female mice is caused by Carbon nanotubes [104]. Ability to enter into vital organs due to their small size, physical properties and heavy metal toxicity can result in toxic responses. There is no present evidence showing specific nanomedicines induced toxicity in humans. Magnetic nanoparticles used for thermal ablation have been revealed to be retained in the urinary tract and cause treatment-related illness [105]. However, several studies have shown that nanomedicine will not cause any toxicity in animals.

5.1 Toxicity of nanomedicines

Nanomedicine integrates with organic particles immunologically and inducing alteration in their structure and capacity which may cause carcinogenesis and hypersensitivity. Due to the very small size of nanomedicine, it may diffuse into the organic matter and localize inside the body cells, tissues, and other subcellular compartments. Toxicity (Carcinogenesis and hypersensitivity) may be induced by the accumulation of organic components and the overcrowding of these biological compartments.

5.2 Oxidative pressure

Oxidative Stress can cause the generation of the reactive oxygen species (ROS) within cells. ROS can be radical (hydroxide radicals, nitric oxide) and non-radical (singlet oxygen and hydrogen peroxide). Our body can tolerate a small amount of ROS [106]. But organic pressure present in the body which integrates attack by pathogens and nanoparticles leads to the escalation of ROS. The proliferation of ROS in the body can cause a fatal effect known as xidative pressure [107]. After degradation of nanomaterials, generation of ROS is very common, however, its toxicity and fatal effect depends on the nanomaterial type [108].

5.3 Immunogenicity

Nanomaterial can induce immunogenic reaction due to the adjustment of the cell-interceded reaction [106]. It is an effect where our body cells treat nanomaterial as foreign particles and increase cytokines or protein signaling response (e.g., TNF-α and chemokine IL-8). The immunogenic reaction can be assessed by ELISA or the microbeads examine technology [109].

5.4 Genotoxicity

Coordinate physical (i.e., intercalating twofold DNA helix) or may be compound connections of nanomaterial with the DNA can result in Genotoxicity [110]. Oxidative pressure is stimulated by the complexation of Nanomaterial with the DNA which is further helpful in the production of ROS and RNS (Reactive nitrogen species) that can harm the DNA. The effect can be breakage of DNA double strand, DNA discontinuity, and suppression of DNA capacities. The analysis of genotoxicity embraces the DNA transformation, location of DNA fracture, breakage of DNA double-strand and alteration in the direction of DNA replication and quality [106, 111].

5.5 Nanomedicines effect on environment and other species

Production of nanomedicine generate tons of materials which is exposed to the environment. Mostly nanomaterials enter into the environment by the disposal of waste. Unacceptable release of these contaminants is becoming a worldwide problem and nanomedicines may accelerate this issue. Nanomedicines will be able to travel for a long duration of time in the environment and contaminate groundwater, soil, river system, and enters into the food chain. Nanomedicines may accumulate and interact with other contaminants and cause toxicity [102].

5.6 Problems in scale-up technology

Main problems during scale-up production from milligram to a multigram level under GMP are reproducibility, stability, sterility, and non-pyrogenicity. At the same time, consideration of critical features is as per GMP design with the maintenance of continuous quality control, risk evaluation for specifications for excipients, manufacturing process, intermediates as well as finished products [112]. The main reason for failure in the translation of any preparative method from small batch size to large batch, i.e., the laboratory scale to an industrial scale with limitation in the small-scale production. A well-made scale-up technique will guarantee the quality of the nanomedicine and cost-effectiveness with the timely product launch. Exclusively, emulsion-based method, as well as nanoprecipitation method, are available for formulating nanomedicine and are well reported in the literature, which is supported because of scale-up aspects in industrial

manufacturing. Characteristics of the nanoparticles, for example, particle size, process residual material, encapsulation, colloidal stability, and surface morphology are mainly affected by scale-up production. Scale-up production from the laboratory scale to the industrial scale using the emulsion-based method, did not alter the encapsulation efficiency. Particle size is decreased by increasing the impeller speed and agitation time. Particle size is also decreased with the decrease in the concentration of the polymer [102].

6. Morals in nanomedicines

Expenditure is very high in research and development associated with nanomedicines which makes nanomedicines expensive and sale of the nanomedicines do not pay off for the research cost invested over many years. Nations which are developed, are endlessly trying to improve the existing treatment strategies by making newer advancements in nanomedicines. In underdeveloped countries expenditure in nanomedicines is sparse and also expensive for the poor. Thus, there is fright that nanomedicines will build a gap between rich and poor. Nano ethics lag behind due to the deficiency of the policies related to the funding and educational institutions. The distinction between therapy and enhancement is another ethical problem. For example, respirocytes are artificial oxygen boosted red blood cells that confine a reservoir of oxygen. These respirocytes are used by heart attack patients so that they can receive oxygen easily and continue breathing until they get proper medical treatment. But athletes administered these respirocytes for bodily enhancement and for boosting their performance. So, it became the main problem in nano ethics for distinguishing between therapeutics and overuse. Even though nanomedicines are helpful in enhancement as compared to the conventional medicines, we should consider the proper therapeutic use before promoting research in this area [113].

Due to the small size of nanomedicines and breakage of nanomedicines in the body, it will aggregate and result in toxicity. So short-term or long-term toxicity studies should be performed during preclinical and clinical studies to assure the safety and efficacy of the nanomedicines. The investigator must take one agreement from the participants of the clinical studies. Participants should be knowledgeable about the procedure, risks, benefits of the study and duration [114]. It is unethical if the investigator involves the person who has not signed agreement or informed consent form. Nano chips are useful in detecting the abnormalities in the cancerous cells or genes which are blameworthy for the disease. Nano chips are also used in prediction of the existence of the disease in future by analyzing DNA. Future prediction of the disease can generate panic, anxiety, and increase fear of illness results in psychological harm. Chip-based nanotechnology is also explored in pain management. Silicone-based chips incorporated with various thousands of monodispersed nanomedicine-based chips have been developed and incorporated in the form of implant. These implants can provide control release of drugs and

biotechnological-based products. Adoption of nanomedicines in hyper diagnosis and hypnotherapy results in unnecessary information which is detrimental to the person so it should be prevented [115, 116].

7. Conclusion

Nanomedicines is an efficient field for pain management and general medicine. Now there is a need to rapidly transfer these technologies to the clinical use while keeping safety and efficacy of nanomedicines that will improve the quality of life and decrease the pain. Further, we need to decrease and prevent the attack of microorganism and bacteria on the implanted devices, i.e., biofouling on the implanted devices as well as increasing the responsiveness and biosensing capacity of the implanted devices. Additional efforts should aim towards the rapid and effective treatment of the breakthrough pain. Further, we need specific therapies to treat postoperative acute pain that will result in chronic postoperative pain syndrome so there is a need to prevent this chronic postoperative pain syndrome [117]. There is no available device to accurately and objectively measure pain so there is need of development of tool or device to accurately and objectively measure pain so that physician will use the baseline to treat pain and also use these measurements to evaluate the effectiveness of the therapy. For nerve repair and regeneration, research in tissue engineering should continue. As well, for repairing nerve and spinal cord injuries, inorganic substrate neural cell interaction should continue. Additionally, there is a need for imaging modalities with nanoparticles which will offer a view of the neurons, neural pathways, and their substructures with the utmost possible definition. Nanoparticles of these groups got energized by external radiation or radiofrequency energy after localization in those afferent pathways and provide nanoparticle-mediated neurolysis. Recognizing and overcoming hurdles early in the development process will deduct the time needed to transfer the technologies from idea to the clinical use [118].

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CHAPTER 16

Advances in transdermal delivery of nanomedicine

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Abbreviations

DDSs drug delivery systems
MN microneedles
SC stratum corneum
TDDS transdermal drug delivery system

1. Introduction

Transdermal drug delivery system (TDDS) penetrates the drug molecule across the layers of skin into systemic circulation for distribution. TDDS offers painless drug administration with high patient compliance and also helps to avoid first-pass metabolism. It provides sustained delivery of the drug molecules as well as reduce several drawbacks like degradation of the drugs within the tract of intestines because of enzymes, level of drug concentration, troubles in the injections, etc. associated with oral, intervalvular, hypodermic ways [1]. These drug delivery systems (DDSs) continuously deliver drugs which have short biological half-lives and it helps to eliminate pulsed entry into systemic circulation which sometimes causes unwanted side effects.

2. Anatomy of the human skin

To design an ideal TDDS it is essential to understand the skin anatomy and its implication. Skin plays an important role in protecting our body. That is the largest organ of the human frame and covers a surface location of about 2 m^2 and receives about one to one-third of the blood movement via the body. It serves as a permeability barrier in opposition

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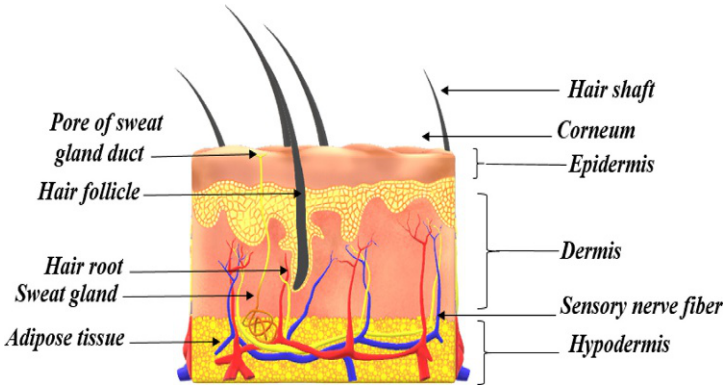

Fig. 1 Anatomy of the skin for designing the transdermal drug delivery system.

to the transdermal absorption of numerous chemical and biological factors. It is the outer covering of the body with a thickness of few millimetres. However, the skin is a most complicated obstacle to the entrance of small quantities of drug to penetrate over a period of time. Skin is mainly made up of three main layers: the epidermis, dermis, and hypodermis (subcutaneous layer) (Fig. 1). Other features of skin related to anatomy are appendages, along with hair follicles and related sebaceous glands (pilosebaceous devices) and sweat glands (eccrine and apocrine glands). Drug molecules in touch with the pores and skin surface can penetrate by way of three different ability pathways, those are through the sweat ducts via the hair follicles and sebaceous glands or directly throughout the stratum corneum. The epidermis may be a constantly self-renewing, stratified squamous epithelium casing the whole outer surface of the frame and in most cases composed of two parts: the residing or viable cells of the stratum germinativum (viable epidermis) and, therefore, the dead cells of the corneum are commonly mentioned because of the stratum corneum. The multilayered epidermis varies in thickness, counting on cell size and, therefore, the number of cell layers of the epidermis, starting from 0.8 mm on palms and soles down to 0.06 mm on the eyelids [2].

The stratum corneum is the outer most layer of skin (Fig. 1). It is nearly 10 mm wide when parched and expands frequently when completely moisten. It has around 15–20 shields of corneocytes made up of keratinized and dead cells which makes it malleable yet approximately impenetrable. The corneum is an important blockage for insertion of the drug in the skin. The construction of the corneum model resembles a wall-like structure [2].

The viable epidermis is situated beneath the corneum layer (Fig. 1) and differs in width, i.e., on the eyelids, it is 0.06 mm and, on the palms, it is 0.8 mm. Moving inside, it contains layers: stratum spinosum and stratum basal. Within the basal layer, constant

renovation of the epidermis is done by mitosis of the cells and the loss of dead Horney cells from the skin surface is recompensed by this multiplication. The basal layers produced cells move outward altering morphologically and undergo keratinization making the outermost layer of the corneum [2]. The dermis may be a 3–5 mm wide layer and consists of a matrix of animal tissue, along with blood vessels, lymph vessels, and nerves (Fig. 1). The blood supply to the skin plays a major role in regulating the body temperature along with supplying oxygen and nutrients to the skin while eliminating waste and hazardous products. Within 0.2-mm distance capillaries reach to the skin layer and supply drop conditions for molecules which enter into the skin barrier [2]. The blood supply to the dermis keeps the concentration of the permeate in the dermis at a minimum level and the resulting concentration gradient between dermis and epidermis facilitates transdermal permeation.

The dermis and epidermis is supported by subcutaneous fat tissue or the hypodermis (Fig. 1). It is a fat cargo area. This layer provides nutritional support, temperature control, and mechanical safety. It transfers primary blood vessels an nerves response to the skin, which has sensory pressure organs. The penetration of the drug through three layers of skin and reaching to the circulation is important in the transdermal drug delivery while insertion through corneum and retention of drug in skin layer is important in case of topical drug delivery system [3–5].

3. History of transdermal delivery systems

Since the past, humans have widely applied (plant-based) naturally occurring products as target-based DDSs or TDDS against various diseases but the therapeutic effect of these drugs was not much relevant for the complete cure of the disease. Advances in the TDDS offer improved efficacy in comparison to conventional pharmaceutical dosage forms. In order to strengthen TDDS absorption, new methods have been invented, progressed, and registered. Year-wise growth of TDDS is briefly mentioned in Table 1. The very first transdermal system (Transderm SCOP) got the approval of the FDA in 1979 for the anticipation of vomiting and nausea along with ravel particularly by sea [6].

Homemade remedies used to treat superficial infections included herbal oils, herbal extracts bandaged on to the skin as TDDS are likely to have originated from the Egyptians, Sumerians, Africans, and ancient manuscripts based on ayurveda (Fig. 2A) [8–10]. The most famous formula is a cold cream created by Galen certainly the composition as much similar to the present (Fig. 2B). In the late 15th-century, new transdermal medications having mercurial ointments, are used for treating the Syphilis (Fig. 2C). Then German Pharmacopeia in 1872, produced a compilation in Latin, listed 28 different Emplastrum formulations. These included adhesive products like Emplastrum adhasivum

Table 1 Year-wise growth to transdermal drug delivery [6].

Year	Developer Stepwise development towards the transdermal drug delivery system
1550 (BC) Papyrus fibers	Described more than 800 prescriptions and about 700 drugs having many formulas for medicating skin, included conditions like wounds, blisters, burns, and exudations, etc.
1872 (German pharmacopeia)	Emplastrum adhesive, which contained oleic acid, leas oxide, colophony, Emplastrum, and adhesive agleam
1882 (Malkinson and Rothman)	Outer application of belladonna (e.g., lotion, liniment, and plaster)
1904 (Schenkenbecker)	Lipid soluble substances and further potential systematic absorption of topical products
1963 (Keith and Snipes)	In vivo studies showed incorporation after topical application by evaluating the level of drug in the sample of blood, urine, and faces
1971 (Chandrasekhar)	Qualitative analytical methods were found in substances in blood and urine by observing the change in a collected sample regarding its color, acidity, and density relative to the standard sample
1943 (Holley and van Stennis)	Photometric measurements and amalgamation methods
2000 (Zaffaroni)	Development of a topical product with systematic effects Nitroglycerin used from the ointment to the transdermal patches for angina pectoris
2005 (Stanley)	Use of transdermal clonidine for the hypertension therapy
2006 (Watkinson)	Transdermal testosterone for hypogonadism
2008 (Hoffmann)	The first transdermal patch to reach the market is scopolamine (hyoscine) patch useful for the treatment of motion sickness
2009–2012 (Zondek)	Transdermal fentanyl for the treatment of pain Transdermal estradiol for female hormone replacement therapy
2014 (Yan, Li Chen, Xian Feng)	For transdermal drug and vaccine delivery use of robust fast-dissolving microneedles
2015 (Hua, S.)	Lipid-based nano-delivery systems used in transdermal delivery of drugs and bio-actives
2018 (N. Nagai, F. Ogata, M. Ishii.)	The biological fate of the nanoparticles specifically, size and shape are the most important factors in the transport pathways of TDDS

that contained oleic acid, lead oxide, colophony, etc. meant to produce systemic plaster (Fig. 2D). Zondek conveyed the first quantitative report of medical controlling of systemic conditions by topical application of chloroxylenol (Table 1). He observed that chloroxylenol (Dettol), still present in antiseptic soaps and solutions as an exterior antiseptic today could be effective for treating the infections of urogenital when externally applied as a 30% lanolin ointment (Fig. 2E). A nitroglycerin ointment TDDS is used in the transdermal scopolamine patch which is available in the market [7]. But the

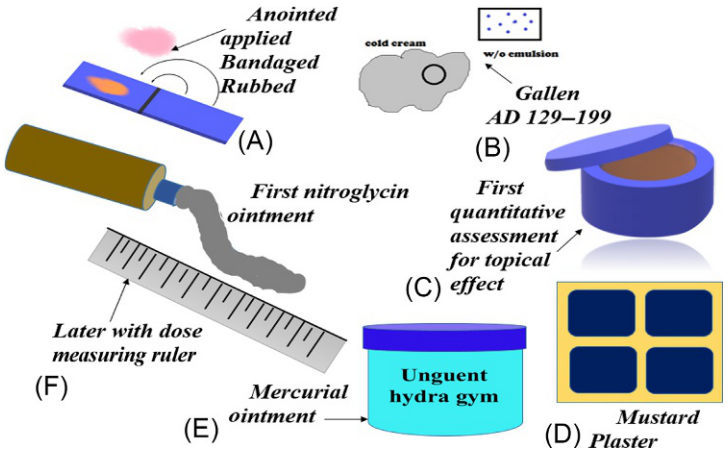

Fig. 2 Pictorial depiction of sequential development of TDDS: (A) products from ancient times, (B) Galen's cold cream, (C) mercurial ointment, (D) mustard and belladonna plasters; controlled dosing of topical products, (E) first quantitative systemic delivery (Zondek's system), and (F) individualized delivery system: mercurial ointment.

application of the enough dose was not a easy process. The amount of nitroglycerin ointment TDDS used in therapeutic trials was determined by use of scale to define the length of ointment ribbon coming out from the ointment tube (Fig. 2F) and having a range from 1.3 cm (1/2 in; 7.5 mg) to 5.1 cm (2 in; 30 mg), externally applied to 232 cm^2 (36 in^2) of the skin of the body [7].

4. Generations of transdermal drug delivery system

The first-generation TDDS were able to deliver drugs up to the stratum corneum. The variations in these drug delivery systems were prepared in the form of sprays, gels, or formulations that could be applied to only superficial infections [11]. The first-generation TDDS set the framework for the development of advanced patches with better efficacy and biocompatibility. This led to wider acceptance of the technology thereby enhancing its marketability. The approach to TDDS is restricted primarily by the barrier posed by the skin's outermost layer called corneum [12]. The second-generation TDDS included skin permeability enhancers which helped in achieving a better drug delivery system. Skin permeability enhancers are entities that help improve the drug delivery by reversibly disrupting corneum structure thus providing the drive for drug transport into the skin. Irrespective of their approach, their design is made to avoid tissue injuries.

In spite of advancements, the second-generation TDDS, using conventional chemical enhancers, iontophoresis, and noncavitational ultrasound, is facing difficulty to achieve the optimum balance between increasing drug delivery inside the corneum and reducing

the injury to the inner tissues of the skin. Hence, these delivery systems of second-generation have key advancement in clinical practice by upgrading localized delivery of smaller molecules, cosmetic as well as dermatological with some systemic applications, which has created an impact of the insertion of the macromolecules [13–16].

The third generation of TDDS composition has made a remarkable impression on delivery of drug. These systems deliver drugs by acting on the stratum corneum [8]. This creates a stronger disturbance in the stratum corneum barrier and has become more effective as TDDS. In this way, advances in TDDS were achieved by designing novel chemical enhancers, electroporation, cavitational ultrasound and currently microneedles, thermal ablation, and nanoneedles [17].

5. Advancement in TDDS

For increasing the performance of restoration for TDDS, extensive efforts had been expended on the improvement of recent methods. They have been widely divided into passive and active technologies (Fig. 3). Using a combination of chemical enhancers in different cases has allowed to improve passive diffusion of small medications [18–21]. Giant efforts have been done in the last few years for the improvement of particle-based

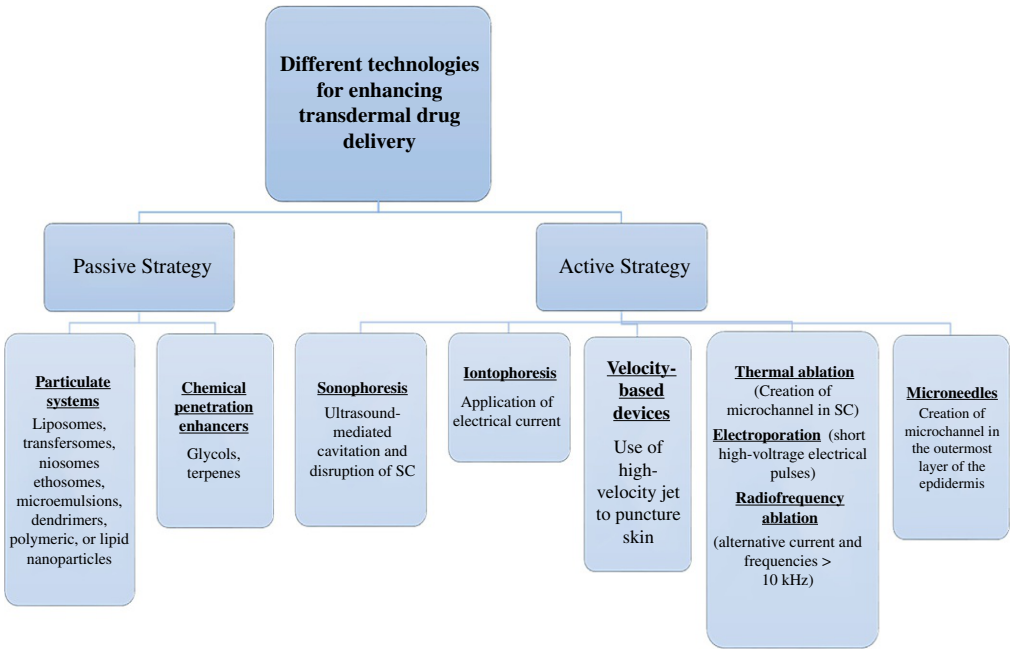

Fig. 3 Flow chart of advancements made in TDDS.

and active technology to renew the shielding properties of the SC. The following description of both passive and active technology for local and systemic transport (Fig. 3), a number of crucial technological advances in these regions are widely mentioned in this chapter.

5.1 Passive strategy

5.1.1 Chemical penetration enhancers

Recognizing the need for skin permeability enhancers, methods of delivery of second generation have increasingly changed to prioritize the chemical enhancers. This outlook may be a logical extension of the traditional pharmaceutical toolbox since it contains creation of new inventions with different chemical exponents. Skin irritation is one of the major challenges that are associated with the delivery even with small molecules [8, 22]. A little group of these enhancers which improves skin permeability without irritation has been successfully started to penetrate the tiny particles impacting on the trouble of hydrophilic compounds to be delivered. Another way is provided by chemical enhancers by drug segregation into the skin for delivery of the drug molecules. These design standards have demonstrated the most desirable chemical systems for boosting drug permeability.

5.1.2 Particulate systems

Along with liposomes, dendrimers and microemulsions are also useful in chemical enhancers with a supermolecular structure that enhances the solubility of drug in the formulation and drug separation into the skin [23, 24]. These procedures have been successful for the better deliverance of particles for outer and cosmetic applications [8]. The clumsiest (difficult to handle) liposome formulation is now in clinical tests for delivery of the insulin [7]. Another TDDS which have been used is prodrugs [25]. By including a cleavable group to the prodrug, the lipophilicity of the drug increases. Such prodrugs make the transfer of one-offs through the skin simple. For example, esters or carbonates which are side chains of alkyl with enzymatically cleavable linkers.

Rather than altering the skin structure, prodrug alters the drug structure to avoid skin irritation [8]. Nevertheless, the advancement of this subject has been limited by means of the complexity of prodrug layout, the applicability of the method to small drugs molecule and the necessity of advantage of Food and Drug Management (FDA) approval of the prodrug as a brand new chemical entity rather than the approval of only TDDS route.

5.2 Active strategy

Active strategy is more advantageous than the passive one. The active modes for skin permeabilization are mostly based on external features that can be physically triggered

such as activation by ultrasound, electroporation, and iontophoresis (which comes under electrically aided methods), power injection or jet injectors (which are based on velocity aided methods), laser and radio frequency heating (which are thermal devices), and also mechanical-aided methods like use of microneedles and tape striping.

5.2.1 Iontophoresis

Iontophoresis has played a crucial role in the delivery of complex drug profiles since decades by propelling the drug with an increased electrical drive [26, 27]. The highly charged drugs are propelled based on their electrophoretic mobility, whereas the weakly charged and noncharged compounds are transported by the differential gradient caused by electrostatic flow of water [28]. Rather than changing the barrier of the skin iontophoresis applicable for smaller particles can carry a charge and some big molecules till a few thousand Daltons. Iontophoresis has an advantage over other technologies by being controllable with the help of a microprocessor as and when required. This modulation ability of iontophoresis has enabled TDDS to deliver complex drugs. However, the proportionate relationship between the current and delivery is severely limited by several factors [29].

Current applications are highlighted and guided by the strong and weak points of the power of iontophoresis that has better delivery control over the drug dose as it measures the quantity of the delivering charge to the skin (i.e., objects of current and time) [26, 27]. Iontophoresis is now clinically used to rapidly deliver lidocaine for local anaesthesia, extracting glucose from the skin for pilocarpine and hyperhidrosis treatment (i.e., excessive sweating) and glucose monitoring to induce perspiration as part of the CF diagnostic assay [30]. A wearable triboelectric nanogenerator (TENG) is very useful for the motion sensor and strength harvester which may change biomechanical variations into power for iontophoresis without saved-energy power assets, at the same time soft patch of hydrogel along with electrodes is designed to permit noninvasive iontophoretic TDDS. Despite the fact that the transport charge is not managed through this low-cost opportunity, overall drug delivery is controlled because the overall rate transferred throughout the skin is limited by battery potential.

5.2.2 Ultrasound-based approaches (sonophoresis)

A skin permeation enhancer is firstly known as ultrasound TDDS when physical therapists found that massaging anti-inflammation agents using ultrasonic heating probes onto the skin thereby increasing the effectiveness of the system. Although some have speculated that ultrasound-related pressure gradients and oscillations may serve as a drive for drugs to penetrate the skin, corneum lipid formation is known to be the dominant effect (Fig. 3). The consequences of noncavitational ultrasound on skin permeability are generally limited to increasing small, lipophilic compounds. Under different conditions, ultrasound also does not produce cavitation bubble activity [31]. This is also limited

by the heating of connective tissue that does not target the corneum and damages the deeper tissues [32]. Different theories endorse that the technique of sonophoresis (SP) is assigned to microstreaming glide near pores and skin, which puts shear stresses to stretch the SC and to form passage for transdermal transport. Thermal results of ultrasound have impact on sonophoretic skin by increasing the diffusion coefficient of the drug and permeability coefficient of the skin.

5.2.3 Cavitational ultrasound

In continuation to warming, the ultrasound is also called to get cavitation, means formation and oscillation sometimes the collapse of bubbles within field of ultrasonic pressure [8]. Cavitation is produced within certain limits such as lower frequency ultrasound which makes it different from the heating of ultrasonic or imaging devices. The possibility for TDDS is that bubbles of cavitation accumulate the energy of the ultrasound, thereby enabling focused effects in place of bubble activity. Because the bubbles are difficult to grow into densely packed tissue, the ultrasound prefers cavitation in the connecting medium between the transducer and the skin (e.g., a hydrogel) [33]. The predicted action of cavity ultrasound is that the bubbles oscillate and fall on the skin surface, creating localized shock waves and liquid micro-jets operating in the cornea. It disturbs the layers of the stratum corneum lipid and thereby increases the penetration of the skin for several times rather than damaging the inner tissues. Cavitational ultrasound is not supposed to contribute a required driving force for transportation of the molecules.

Already it is accepted that cavitational ultrasound promotes lidocaine across the skin and has been studied deeply in animals for the supply of insulin, heparin, tetanus toxoid vaccine, and other compounds [33]. Ultrasound has not been used for the extraction of extracellular fluid like glucose for monitoring of diabetes and extending skin penetration by small molecules and macromolecules up to tens of kilo Daltons [34]. Lasers can similarly enhance skin permeability by the associated shock wave mechanism [35].

5.2.4 Thermal ablation

TDDS providing heat to the skin layer for some degrees for microseconds to milliseconds transfers heat in the skin without permitting the propagation of heat to the viable tissue [36, 37], which prevents tissues from harm and pain. It may include the swiftly vaporizing water in the corneum having the result of significant growth enabling small-scaled craters across the skin layers [8]. More modern studies propose that temperatures properly above the boiling factor of water are needed and that other approaches consist of tissue combustion [38]. TDDS skin heating has been achieved using ohmic microheaters and radio-frequency ablation [39].

5.2.5 Microdermabrasion

Microdermabrasion is the most widely used method for transplanting and carrying skin tissue for the purpose of cosmetics in TDDS. This rubbing mechanism, known as fine-grained sandblasting, has been shown to extend skin penetration to drugs involving lidocaine and 5-fluorouracil, indicating potential uses in TDDS [40]. Delivery of vaccine across the skin layer is smoothened by the use of skin abasing sandpaper. In preliminary studies on delivery of vaccines to animals, strong immune response has been observed to several vaccines when they were administered together with a potent subunit (i.e., heat-labeled enterotoxin of *Escherichia coli*). More recently, the vaccination test on the humans have shown traveler's diarrhea and influenza.

5.2.6 Microneedles

Microneedles are microscopic needles in size they are 150–1200 μm long, 50–240 μm wide, and have 1–25 μm tip thickness (Fig. 3), and they have been shown to extend the transdermal instability of compounds with large molecular mass, by many folds [43]. Microneedles are minimally nosy systems, makes small holes on the skin, which enters into the stratum corneum to conquer its shielding characters. This makes a long range of drug molecules enter and spread easily into the skin layers, and hence it becomes more useful in a huge variety of scientific applications consisting of the extreme osteoporosis, diabetes, and vaccination for influenza. Many recent developments in design and creation at microneedle are worthy to note. Original fabrication techniques consisting of the clean chamber-based sculpture of structures of silicon base have shifted to minimum manufacturing cost methods; microneedles made by metals and polymers are normally found in all kinds of FDA approved assets parent formulas [8].

As per the methodology the simplest way to permeabilization of stratum corneum is to make skin porous by use of very small needles. Over the past few years, the development of microneedles as the delivery of the drug through the skin is new operational sensing systems (NOSY) [42, 43]. The staring concept of microneedles arrangement for TDDS was filed in 1971 in a US Patent and it was supported by the formation of small holes of nearly 1 μm in the skin by which drug molecule penetrated easily without experiencing pain [44]. Henry et al. demonstrated the first proof of phenomenon in 1998 for the asset which improved the delivery of the drug across the skin [45].

Typically, microneedles have been categorized into four different designs: solid, coated, dissolving, hollow, and hydrogel forming.

- *Solid MN:* Simple solid MN having external drug reservoir like a patch, cream/gel, solution, etc., is useful for the pretreatment which leads to the growth of permeability of the skin all inclusively, as mentioned in Fig. 2A. Also solid MNs having the homogeneous solution of the drug in the polymer through the drugs penetrate into the skin (Fig. 4).

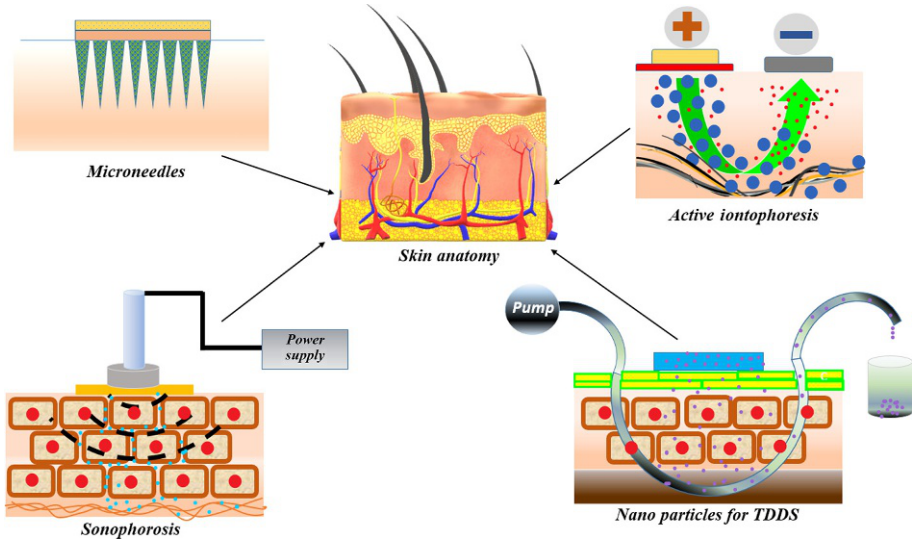

Fig. 4 Advancement in the TDD.

- *Coated MN:* The MNs which are coated with solution of the drug or drug diffusion covering. When small structures are covered (coated) by formulations of drugs the coated MNs are formed. The quantity of drug to be charged in the coated MNs is totally based on the width of the outer layer of the drug formulation and the dimensions of the needle which is very small. Accumulation of the drug formulations takes place after dissipation of the material having drugged into it (Fig. 4).
- *Dissolving MN:* The manufacture of dissolving MNs is done with ecological polymers by loading drug formulation into the polymeric substance (Fig. 4). The advantage of this MNs is such simple that no removal of MNs take place after penetration into the skin [41]. The breakdown of the polymeric substance inside the skin layers and balancing the release of the drug makes it most improved medication therapy for the facilitation of the patients. The most common problem faced by this process during the development of the MNs is the distribution of the drug effectively into the polymeric needles which makes the combination of polymer and drug step much crucial and difficult. In this therapy, most favorable polymeric substance used is PVP as its low toxic nature and stability.
- *Hollow MN:* These are vacant MNs which can be packed by drug formulations. Hence it is useful in the penetration of the compounds like oligonucleotides, proteins, and vaccines which are having large molecular weight [41]. The drug directly gets into the epidermis layer of the skin after insertion into the skin. The penetration rate and the pressure of releasing of the drug molecules is also controlled in case of delivery of large molecules of the drug. These MNs are well sustainable for insertion of huge

dosage since drug-containing capacity of the needle is more. The important requirement for this is consistency in the flow rate of the drug [46]. The flow rate may increase with the increase in the holes in the MNs but the strength and sharpening will decrease. For sharpening the MNs and the strength, the metal covering may be applied on the MNs. The supplement of the drug to the skin is shown in Fig. 2D. The hollow MNs have drug inside the vacant cavity into the needles. Alternatively, pharmaceutical formulations can be coated or incorporated into microneedles to speed up or control peptides and vaccines within the skin. The application of metal or Silicon MNs may cause skin problems. Coating MNs supply very less amount of the drug and its coating is also a difficult process. The MNs made by polymers dissolute early in the skin when it comes in contact with the water and results in a large amount of supply of the drug or another option is the use of hydrogel-forming MNs.

- *Hydrogel-forming MN:* The development of different of MNs has led to the formations of the delivery systems which are based on the use of hydrogel (Fig. 4). It consumes the extracellular fluid to maintain the balance between circulation in the skin layers and the patch reservoir [48]. The arrangement of the hydrogel-forming MNs is given by the Donnelly et al. in 2012 by the use of watery combination of the polymers which suspended on the small silicon molds which are drilled by laser [47]. The gel is kept in the molds for 24 h at 80°C and then the gel layer is taken out from the small silicon molds forming the hydrogel MNs. This gel suspension layer MNs is based on the needle arrangement and combination used for TDDS.

The arrangement of hydrogel MNs is formed by the polymers which have cross-linked structure, silicon materials, and metals too. These structures are mostly eco-friendly and biodegradable polymeric material like PVP (polyvinylpyrrolidone) which is accepted worldwide for the formations of MNs [47]. It is easy to manufacture variety of patches and cleaning and removing out of the skin layers. This developed method favors the penetration of the drug molecules without any limitations and conditions of amount of drug to be inserted and the strength of MNs is based on the cross-linking arrangement of the polymeric substance used to form the hydrogel MNs. The invention in TDDS is enlarging the potency and types of drugs to penetrate by using TDD. It is also useful in the insulin delivery, vaccine delivery, and anticancer medications, and many other therapies of anti-inflammatory and antihypertensive medications [49] (Fig. 5).

These MNs are useful for the delivery of pulsatile and injectable by using electrical assets. Enlarging MNs are also prepared by the use of PVA-dextran, PVA, and hydrogel of gelatine [41]. The enlarging hydrogels when it comes in contact with the internal fluids, crack the tips of the MNs, which allows them to last inside the body for a long time which affects the delivery of the drug [49].

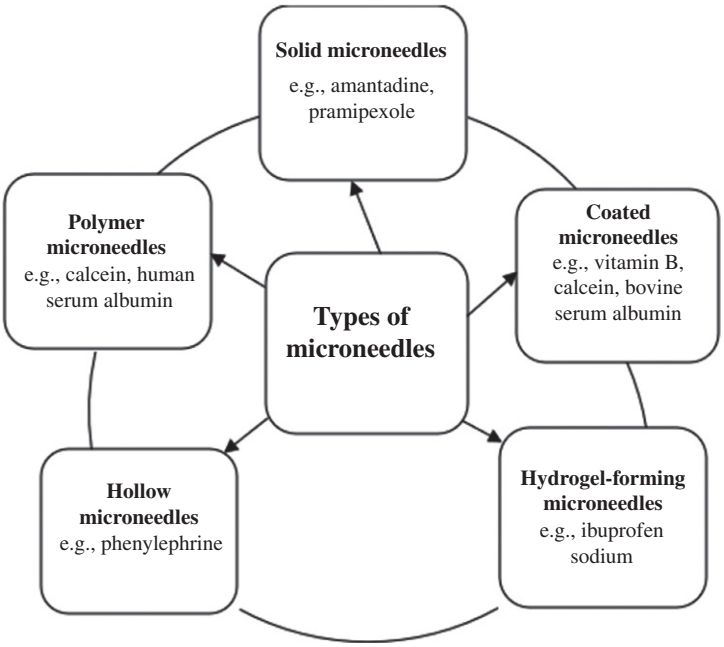

Fig. 5 Schematic representation of different types of microneedles with drugs.

5.2.7 Nanoneedles

They are in the scale range of nanometers, with a diameter of around 50 mm. It is necessary to ensure the reliability of all the individual components and proper needle functionality allowing the appropriate insertion. The characteristics of being small in size, specific in nature for insertion, and balanced releasing of the drug molecules is important for nanoneedles too [46]. Nanoneedles may be used as electrochemical assets and as optical biosensors for testing cellular conditions, activating some forms of biological sequences, and investigating the impact of nanoparticles on cell physiology [49]. The TDDS depends on nanoneedles, which is one of the strongest and developed way for the study of different procedures of biology and the biophysical characteristics at a minor level inside the cells of living beings. This TDDS can be used in the formation of a variety of ways of drug delivery and the penetration mechanism specially spread of the drug into the layers and the circulation. These TDDS can be precisely controlled, monitored, and recorded. Similar to microneedles, nanoneedles can also be developed in different forms, as can be seen in Fig. 4. The drug-carrying hollow needles is activated as it is injected in the layers of skin. On injection, the strong needles produce gaps in the tissue. The addition of a patch then takes place to this region that eventually results in the rapid insertion of drug molecules. Coated needles have a drug-coated base, which continues to function after penetration into the layers of the skin.

The types of polymer needles are dissolving, non-dissolving, and hydrogel-forming needles. The PVP is the most applicable polymer in the manufacture of the nanoneedles [46]. The basic working principle of nanoneedles and MNs is very similar rather than smaller size and experimentally undetectable. And hence this method has greater advantages for pain-free delivery of the drug molecules in the adequate amount. This tends patients to receive medications with tolerance. Nanoneedles are leading in the area of maintaining the balance of drug penetration which is a difficult process for other TDDS.

5.2.8 Transdermal patches

A transdermal patch is an adhesively medicated skin patch used to deliver the doses into the bloodstream through the skin in a controlled manner. Transdermal patches are limited to only some of the pharmaceuticals such as nicotine patch as an example, releases nicotine into the bloodstream to limit the habit of tobacco smoking [15, 50]. Some other uses of skin patches are for the nitroglycerin for angina, estrogen administration for menopause, stopping of osteoporosis after menopause, etc. [51]. Different sorts of a skin patch for transdermal delivery systems have been described.

- Single-layer drug-in-adhesive

The single-layer drug-in-adhesive systems contains temporary liner-layer and backing with a function of sticking to the skin, drug-carrying and releasing effects (Fig. 6).

- Multilayer drug-in-adhesive

The multilayer drug-in-adhesive patch is analogous to the single-layer system but both adhesive layers are capable to discharge the drug (Fig. 3). The multilayer system is different, however, it adds another layer of drug adhesive, usually separated by a membrane. This patch also features a temporary liner layer and a permanent backing.

- Reservoir

The reservoir having the drug gel in the suspension easily disperses into the skin. The drug reservoir systems are inserted in between a rate-controlling membrane (controls drug release) and an impervious backing layer.

- Matrix

The drug reservoir systems are inserted in between a rate-controlling membrane (controls drug release) and an impervious backing layer. This is divided into two categories, i.e., (i) Drug within-adhesive systems (ii) matrix dispersion systems. In the former category, the drug reservoir is made by the drug dispersion into an adhesive polymer followed by the medicated adhesive polymer by solvent casting or melting on an impervious backing layer. The reservoir also contains some unmediated adhesive polymer layers over its top which serves as a cover. In the latter category the drug dispersion is made homogenously during a hydrophilic or lipophilic polymer matrix.

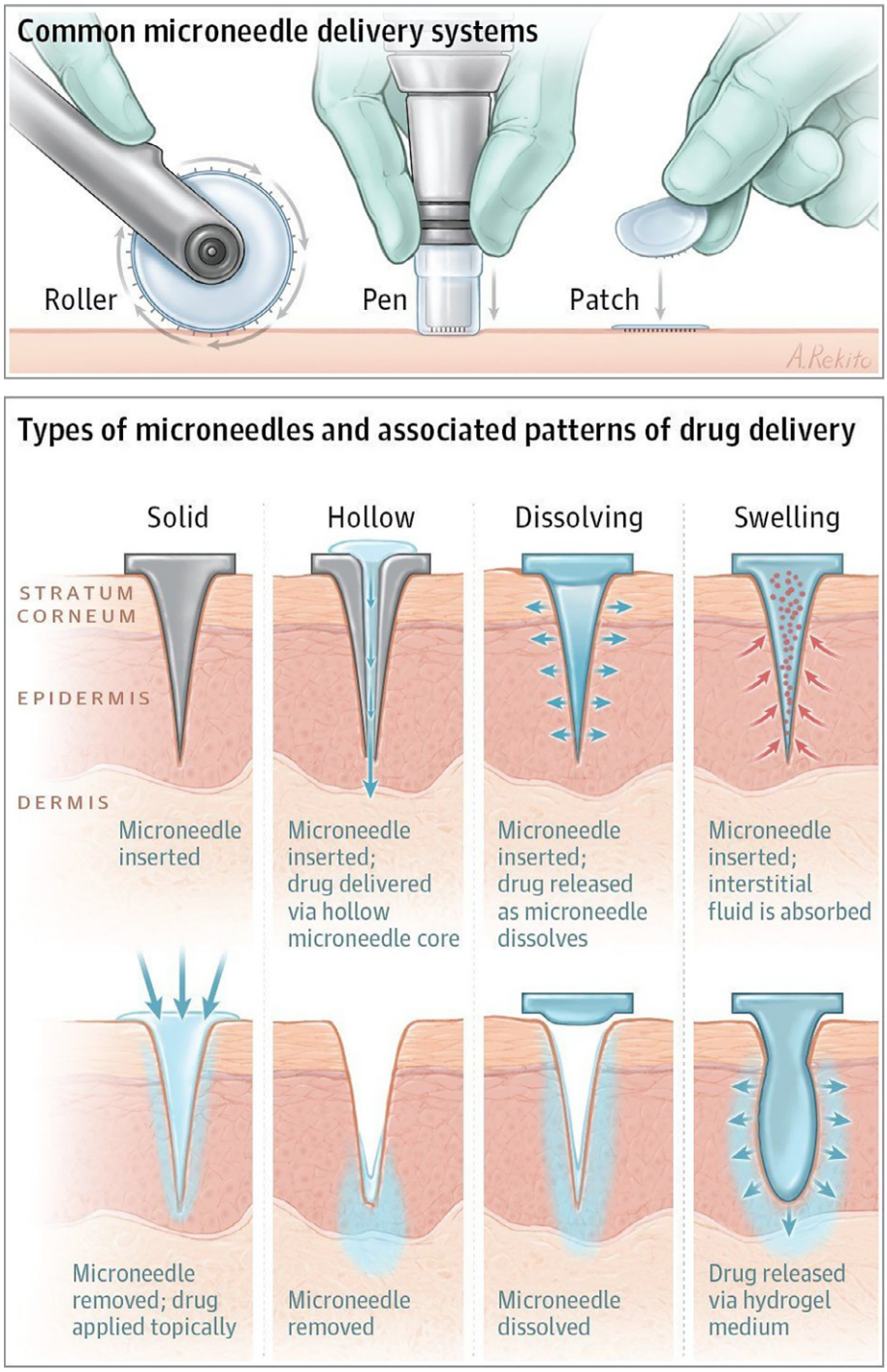

Fig. 6 Types of microneedles and associated pattern of drug release. *Adapted from https://images.app.goo.gl/udRMyKcBNarxbSRR8.*

5.3 Vapor patch

The adhesive layer of a vapor patch serves not only to stick the patch to the skin but also releases vapor. These vapor patches can be used to release essential oils. These products are new to the market and there are vapor patches that contain nicotine to reduce the urge to smoke.

5.4 Micro-reservoir system

Micro-reservoir system contains a drug reservoir and a matrix-dispersion system (Fig. 6). The drug reservoir holds the drug in the form of a gel or solution or embedded into a solid matrix. The embedded solid matrix is stabilized by using cross-linking agents. The drug reservoir is sandwiched between an impermeable layer on one side and a porous controlled release layer on the other side. A polymer adhesive layer is applied to the controlled release layer [52] (Fig. 7).

6. Factors affecting transdermal permeation

6.1 Biological factor

- *Skin conditions:* Although the skin is a natural barrier, some chemicals like acids and bases can penetrate through the skin. Several solvents can disturb the dense structure of stratum corneum. Solvents like chloroform and methanol can create artificial shunts

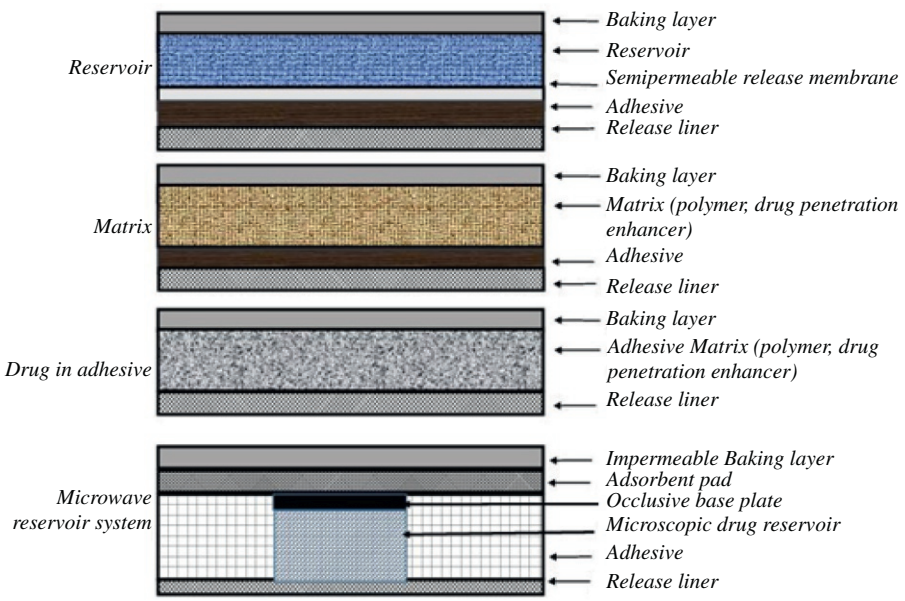

Fig. 7 Different Types of TDDS patches (vertical section).

by removing lipid fraction of the skin. Drug molecules can pass easily through these shunts.

- *Skin age:* The skin of adults and young person's is more permeable compared to older person's skin even though the structure is the same. Children's skin exhibit more surface area per unit weight so boric acid, steroids produce severe side effects.
- *Blood Supply:* Transdermal absorption is affected by changes in the peripheral circulation.
- *Regional skin site:* The extent of transdermal drug penetration depends on the site of skin because the composition of skin (thickness, density, and nature of corneum) varies from site to site.

6.2 Physicochemical factors

- *Skin hydration:* Hydration state of the skin greatly influences the permeability. Moisture increases the skin permeability so humectants are used in transdermal drug delivery.
- *Temperature and pH:* Temperature variation greatly alters the permeation of drugs. The dissociation of weak acids and bases depends on the pH of the medium. The quantity of undissipated drug (weak acid or base) determines the concentration of drug that is absorbed. So controlling temperature and pH is very important in transdermal drug delivery.
- *Drug concentration:* The flux of a material across a membrane depends on the concentration gradient across the membrane. So to get a significant flux across the skin, the concentration gradient should be high. The flux depends on the concentration of drug on the surface of the skin.
- *Partition coefficient:* Partition coefficient is the ratio of concentration of a material in two different phases. If the partition coefficient of a drug is high, it will be trapped by lipid portion of skin, if the partition coefficient is low it will not penetrate through the skin. So optimal partition coefficient is required to achieve good absorption of the drug.
- *Molecular size and shape:* Large molecular size or high molecular weight hinders the absorption of the drug through skin. Small molecules can permeate faster than larger molecules.

6.3 Environmental factors

Environmental factors also influence the efficacy of TDDS. Those factors include sunlight, air pollution, and cold season [57].

- *Sunlight:* When exposed to sunlight, the walls of blood vessels in the skin become thinner and results in bruising. Pigmentation of the skin is the most common consequence of sun exposure.

- *Cold Season:* The skin becomes dry and itchy when exposed to cold environment. Drinking plenty of water and protecting the skin from cold environment by good quality moisturizer will prevent cold-induced dryness.
- *Air Pollution:* Clogging of pores on the skin by dust and dirt leads to accumulation of bacteria in the pores which cause acne and spots. This causes a detrimental effect on the drug delivery through skin. Chemical pollutants in air can alter the natural physiology of skin which leads to problems in drug delivery.
- *Effect of warmth on Transdermal patch:* Heat-induced high absorptions in TDDS. The patient should avoid exposing the TDD patch site to an external heat source. Even high blood heat can increase the transdermal delivery of drugs. In this type of situations, the patch should be removed immediately and should be stored in their original packing and place in a cool, dry place until they are able to use.

7. Advantages of TDDS over other routes of administrations

Due to various advantages the TDDS are the best alternatives to the parental and oral DDs. The disadvantages of oral drug delivery systems like enzymatic digestion attack, drug fluctuations, drug degradation, and hydrolysis, first-pass hepatic metabolism, and gastrointestinal irritations can be reduced by the utilization of transdermal patches [53]. These transdermal patches have many medical applications, such as gradual relief in pain, contraception, smoking cessation, osteoporosis, and in certain cases of cardiac disorders also. Transdermal patches capable of delivering various complex drugs are under research consideration. In certain cases, the transdermal patches need to be developed with many physicochemical properties of active and inactive components according to its suitable usage. So, research works consisting of advanced physical and chemical approaches are under consideration for the development of advanced skin patch development. They avoid the difficulties of gastrointestinal absorption of drug (caused by the gastrointestinal enzymatic activities, pH encounter, drug interaction with drink, food, and other orally administrated drugs). They can be the substitute for orally preferred medications when the rout is inappropriate for administration (in case of diarrhea and vomiting). They avoid the drug deactivation by liver enzymes and the first-pass metabolism. They avoid the inconvenience of parental therapy. They improve the compliance over dosage forms which requires frequent administration of dosage. Early release from medications therapy as TDDS patch can take out from the skin rapidly after curation. These are found to be very rapid and simplest techniques in the emergency cases also (e.g., unresponsive, unconscious, or comatose patient) due to their physical presence, features and identifying markings. They can be used for drugs with narrow therapeutic window. It maintains the drug level under a therapeutic window (in-between minimum

effective concentration and side effect apparent level) for a prolonged period. Compared to an oral and intravenous route, the transdermal drug delivery system provides a steady blood concentration by avoiding peaks and valleys in the plasma level.

8. Disadvantages and limitations of TDDS

Several approaches have been attempted to deliver medications and improve efficacy across the skin barrier. TDDS offer wider range of advantages over traditional topical formulations, it is still suffering from limitations. The residue must have suitable physicochemical properties to penetrate through the corneum, and it is impossible to deliver high dose (>10 mg per day) [54]. Another limiting parameter increases percutaneous absorption. To develop a transdermal product, we need to consider the variation in the function of skin from one place to another, from person to person, and variation with age. These variations make it difficult to develop a pharmaceutical transdermal product [55]. It is not possible to deliver ionic drugs using TDDS, which is a major limitation [2]. It is not possible to attain high levels of drug in blood by TDDS. Local irritation is likely to occur at the site of use. If the drug or formulation causes irritation to the skin, it has undergone modification with reduced cytotoxicity or has to be retracted from the market. A drug which is delivered by TDDS should have adequate hydrophobic and lipid solubility (log P (octanol/water) = 1 to 3) to penetrate the corneum. Drug molecules with molecular weight >500 Da are usually difficult to deliver through the stratum corneum. Drugs with very low or high partition coefficients fail to deliver in the bloodstream. High-melting drugs are often delivered in this way due to their low solubility in both water and fat [56].

9. Regulations and reality of TDDS

Most of the transdermal drug delivery systems are designed for self-administration at home. The TDDS to effective administration of drugs having criteria like patient-convenient and cost-effective measures, ensures the clinical impact. Various patient-convenient measures can be taken by the use of chemical (can be integrated into small and inexpensive patches) [57, 58] and physical enhancers (more effective for vaccine and macromolecular delivery). These physical enhancers are reusable devices with disposable drug reservoir component, requires electric power (in case of handheld devices), and mostly expensive. But the use of microneedles has more significant advantages as it is very cost-effective and physically invasive [8]. In the current scenario, TDDS are in still clinical trials. Particularly, drugs have low aqueous solubility and high toxic side effects, and these new formulations of drugs for TDDS are often capable of reducing the toxic side

effects of the drug molecules. Although some medicines are regulated by the FDA, there are many programs currently underway in clinical trials, indicating that many nanotechnology-based new drugs are ready to hit the market soon.

9.1 Treatment of Parkinson's disease

Parkinson's disease (PD) is a neurodegenerative disease affecting a large count of population throughout the globe. Losing control over autonomic system affects the lifestyle of an individual. PD becomes more complicated when the affected individual loses quality of sleep due to insomnia, increased frequency of eye moments, nocturia, etc. Currently, there is no permanent cure to this disease, however, better management approaches are available to deal with the disease. Several studies have reported rotigotine (a dopamine agonist) in the form of transdermal patches, which efficiently allows a PD patient to gain proper sleep. In a review published by Rosa-Grilo et al., rotigotine when applied in the form of transdermal patches significantly helps PD patients to recover from several related disorders [58]. The effect of rotigotine transdermal patches has also been well highlighted by James Frampton, on the account of enhancing the health-related quality of life [59].

9.2 Treatment of achilles tendinopathy

The preparation of transdermal patches using glyceryl trinitrate for the delivery of muscle relaxants like cardiac diseases and achilles tendinopathy have been implicated [60]. The glyceryl trinitrate acts as an NO_2 donor which purposefully stimulates the synthesis of collagen in muscles as a result the rate of healing increases. The wide spectrum use of glyceryl trinitrate has been explored [61]. In a systematic review by Assem and Arora (2015), on the applicability of such glyceryl trinitrate base transdermal patches suggest the strong effect of the bioactive agent in reducing pain involving different muscular problems, which had a relative high value in relation to the control. Based on this, the authors suggested more comparative studies with equivocal therapeutic agents at a larger scale (Fig. 8) [61].

9.3 Delivery of antineoplastic agents

Delivery of antineoplastic agents against cancers through transdermal route is an attractive choice to enhance the rate of target-specific drug delivery. The efficacy of the approach is hindered by the resilient nature of skin in neonates and infants but in the case of adults, the mode of delivery has been proved to be efficient. In a study by Wang et al. (2016), microneedle-based transdermal patches containing anti-PD1 antibody was used to treat melanoma cancer. The disbursement of nanoparticles conjugated with the antibody in the acidic

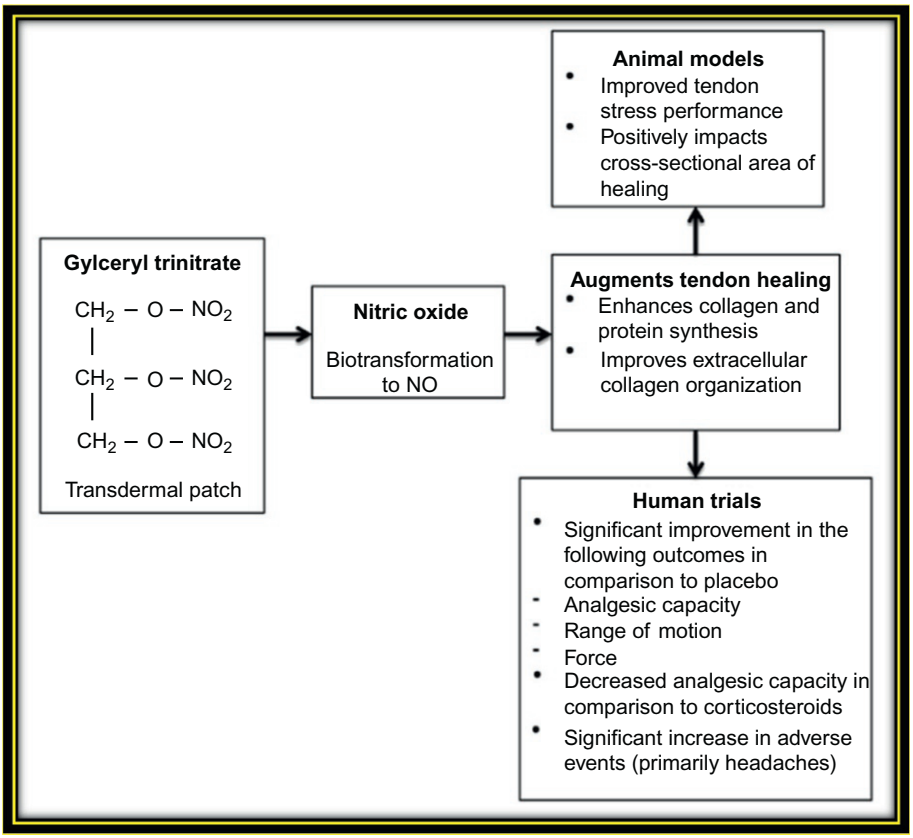

Fig. 8 Application of glyceryl trinitrate transdermal patches. *Adapted from Y. Assem, M. Arora, Glyceryl trinitrate patches: an alternative treatment for shoulder impingement syndrome, J. Orthop. Translat. 3 (2015) 12–20.*

environment enhanced the rate of killing of melanoma cells in the mice [62]. This suggests that incorporation of microneedle in a transdermal patch to deliver antineoplastic agents or immunomodulators can enhance the rate of healing in the case of cancers (Fig. 9).

9.4 Management of diabetes by transdermal drug delivery approach

Diabetes mellitus is a metabolic disease caused by the inappropriate quantitative production of insuling by pancreas. This disease is usually managed by the delivery of insulin either through parenteral or injection. The development of TDDS for managing diabetes has shown promising results as reviewed by Ching and Gupta [63].

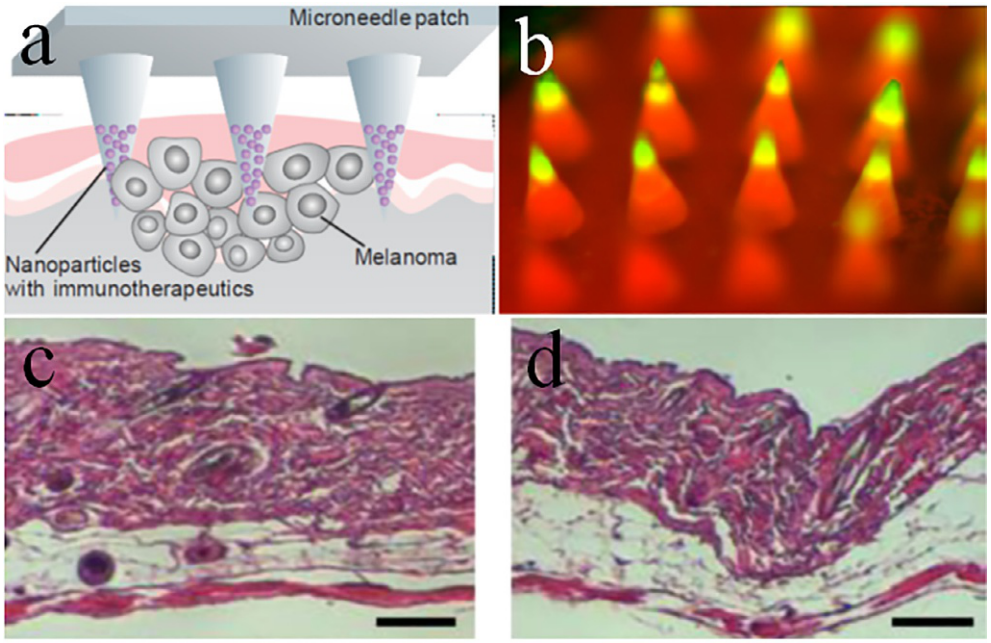

Fig. 9 (A) Pictorial representation of microneedles, (B) fluorescence imaging of a representative microneedles patch that contained FITC-antibody loaded NPs (Scale bar: 200 μm) (C) H&E-stained skin sections administered a MN patch (Scale bar: 200 μm) and (D) surrounding tissues 2-d postadministration (Scale bar: 200 μm). *Adapted from C. Wang, Y. Ye, G. M. Hochu, H. Sadeghifar, Z. Gu, Enhanced cancer immunotherapy by microneedle patch-assisted delivery of anti-PD1 antibody, Nano Lett. 16 (2016) (4) 2334–2340.*

The implementation of several advanced technologies in TDDS to manage this metabolic diseases, microneedles seem to be more efficient than others due to their capacity to penetrate the skin pores while delivering drugs efficiently to control the blood glucose levels [63].

10. Conclusion

This chapter summarizes recent advancement of TDDS. From the history of TDDS to the latest advanced techniques of TDDS that are being used for clinical application, this delivery system offers improved bioavailability of many orally administered drugs to avoid pain due to injections and improved controlled release of drugs. Transdermal drug delivery is the best drug delivery system for macromolecules, supramolecular constructs, and viral particles because of their low dose administration. TDDS targets epidermal longer on cells and dermal dendritic cells which can trigger strong immune responses even at

lower doses. So this drug delivery system is effective for vaccine administration. For example, smallpox vaccine was administered via skin which eradicated the disease worldwide. TDDS provides good control over the distribution of drugs.

The skin, which has long been considered inappropriate for drug delivery, is currently expanding on its physiological significance and potential drug delivery strategies. With the growing popularity of TDDS, device-assisted transdermal delivery provides an alternative paradigm for TDDS in response to the real-time progression of the disease. Since the incorporation of advanced skin linkage formulas is still in the early stages, there is space for future research to develop wearable devices and improve the reliability of the system and their application to assorted disease models. Continued efforts to strengthen the delivery of drugs that are considered inappropriate for administration through the skin (e.g., peptides, nucleic acids, and macromolecules) should also be managed to expand the library of transdermal compatible drugs. However, more development is needed for patient-personalized TDDS. This approach holds the promise of convenient management of lifestyle diseases and a unique paradigm for future health.

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CHAPTER 17

Insulin nanoformulations for nonparenteral administration in diabetic patients

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1. Diabetes mellitus

Diabetes mellitus (DM) is a metabolic disorder caused by either insufficient insulin production by the pancreatic β-cells or the body unable to use produced insulin effectively. DM is a universal health care concern associated with various complications affecting the kidneys, the cardiovascular system, and causing retinopathy and neuropathies [1]. The prevalence of DM has increased dramatically worldwide over the last few years [2, 3]. From 2000 to 2013, the number of DM patients has swelled from 171 to 382 million, and it is projected to rise from 592 to 642 million worldwide from 2035 to 2040 [2, 4]. DM primary forms include types I and II and are both characterized by hyperglycemia [5]. Other types such as gestational DM and genetic alterations of β-cells are less frequent [6]. DM type I accounts for 7%–12% of DM cases. The number of DM type II cases increased by fourfold when compared to type I and is thought to increase even further because of the sedentary lifestyle and lack physical activity embraced by modern societies [7]. As of 2016, records from the World Health Organization (WHO) reported that DM is the seventh leading cause of death globally [8, 9], directly responsible for at least 5 million deaths in 2015 [10]. The total health-care budget allocated for the management of DM in the United States has risen steadily in the last few years [11]. In 2012, the US government allocated $245 million to the health-care budget for the management of DM, which reached $327 million US$ in 2017 [12].

2. Types of diabetes mellitus

DM type I is the consequence of an autoimmune reaction targeting the β-cells and leading to absolute insulin deficiency [13]. DM type II is a noninsulin-dependent type of DM whose development is dependent on an array of genetics, physiological and

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environmental factors including the lack of physical activities, smoking habits, and age [14]. In DM type II, the peripheral resistance to insulin can be due either to the overproduction of insulin by the β-cells or insulin deficiency as a result of the impaired β-cell function [15].

3. Insulin

Banting, Best, and Macleod discovery of insulin in the 19th century has significantly transformed DM into a manageable condition [16]. Insulin is a 51 amino acid protein formed by A and B chains connected through disulfide bonds [17]. Chain A and B are composed of 21 and 30 amino acids, respectively (Fig. 1A) [18]. The hormone is synthesized as preproinsulin, which is then cleaved into proinsulin when the signal peptide is removed during the transition in the endoplasmic reticulum [5]. Proinsulin is then broken down by endopeptidases to form a mature form of insulin. The free C peptide and insulin are packaged as microcrystalline zinc insulin hexamer into secretory granules in the Golgi apparatus and accumulate in the cytoplasm [6]. Upon excitation of the β-cells, insulin is exocytosed and diffused into blood capillaries [5]. A high blood glucose level usually activates the release of insulin. However, some amino acids, like leucine and glutamine, can promote β-cell insulin secretion. Leucine stimulates glutamate dehydrogenase enzyme which catalyzes the conversion of glutamate to α-ketoglutarate. On the other hand, glutamine undergoes a series of metabolic steps to form α-ketoglutarate, which then enters the citric cycle to produce ATP. ATP production is enhanced by the secretion of insulin [13]. Further, alanine and glutamine into blood circulation, act as signals for the secretion of glucagon, which then triggers the secretion of insulin [14, 15].

The insulin receptor is a heterotetrameric structure made up of two extracellular alpha subunits, that binds to the insulin and two transmembrane beta subunits [19]. Furthermore, insulin receptors of skeletal muscles and adipose tissues are dispersed within the cell plasma membrane. The binding of insulin to the alpha subunits, triggers autophosphorylation of the beta subunits [20]. Both the activation of the insulin signaling pathway and translocation of glucose transporter type 4 (GLUT4) are essential for the efficient entry of glucose. The GLUT4 is recycled to the vesicles and dissociated from the alpha subunit of the receptor when the insulin level is reduced in the blood circulation [19, 21].

4. Insulin formulations and analogs

Management of DM initially relied on the insulin extracted from the porcine or bovine pancreas. However, the rising diabetic population and the concern for the development of allergic reactions led to the development of recombinant insulin. A brief history, in

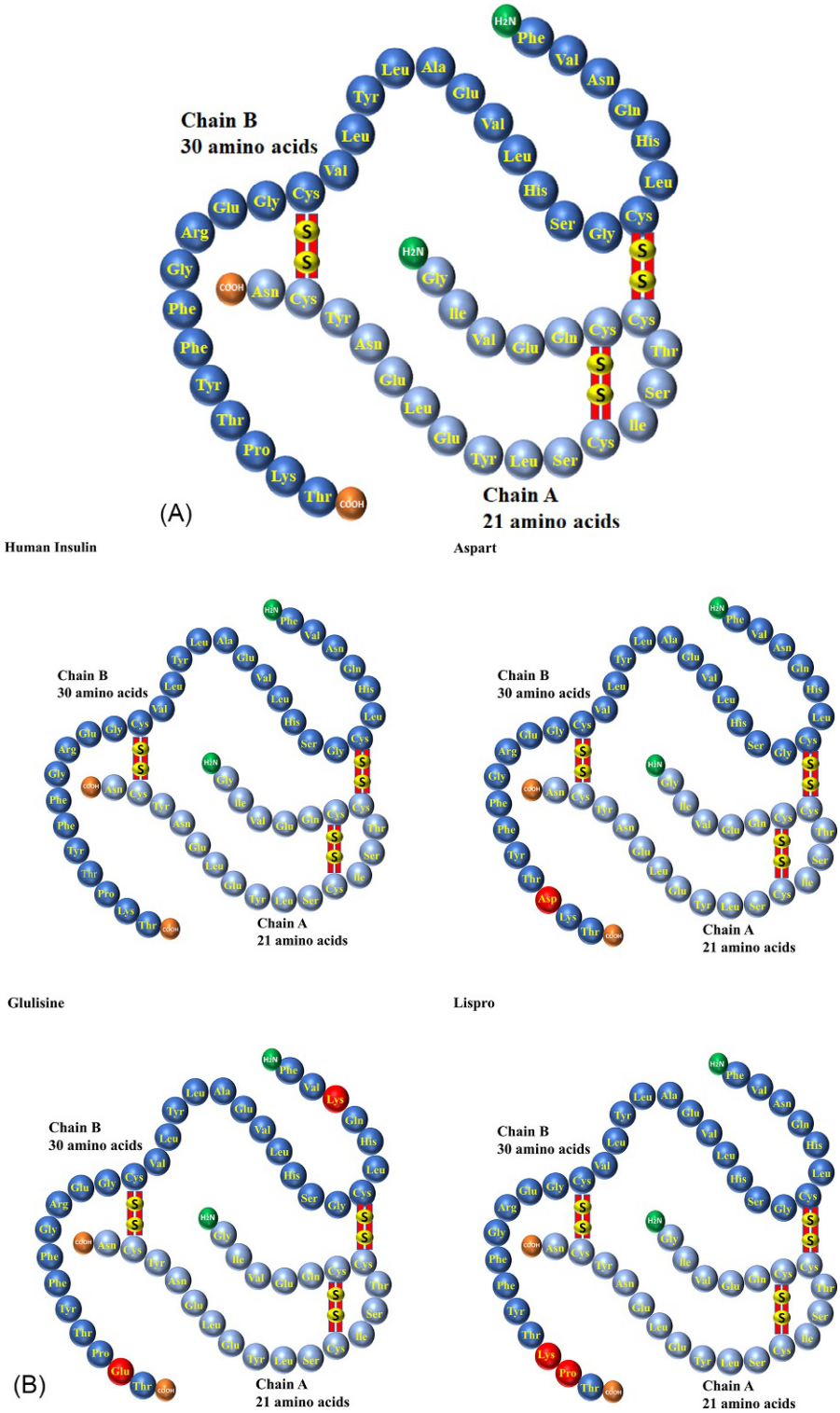

Fig. 1 (A) Structures of human insulin, (B) rapid-acting insulin analogs, and

continued

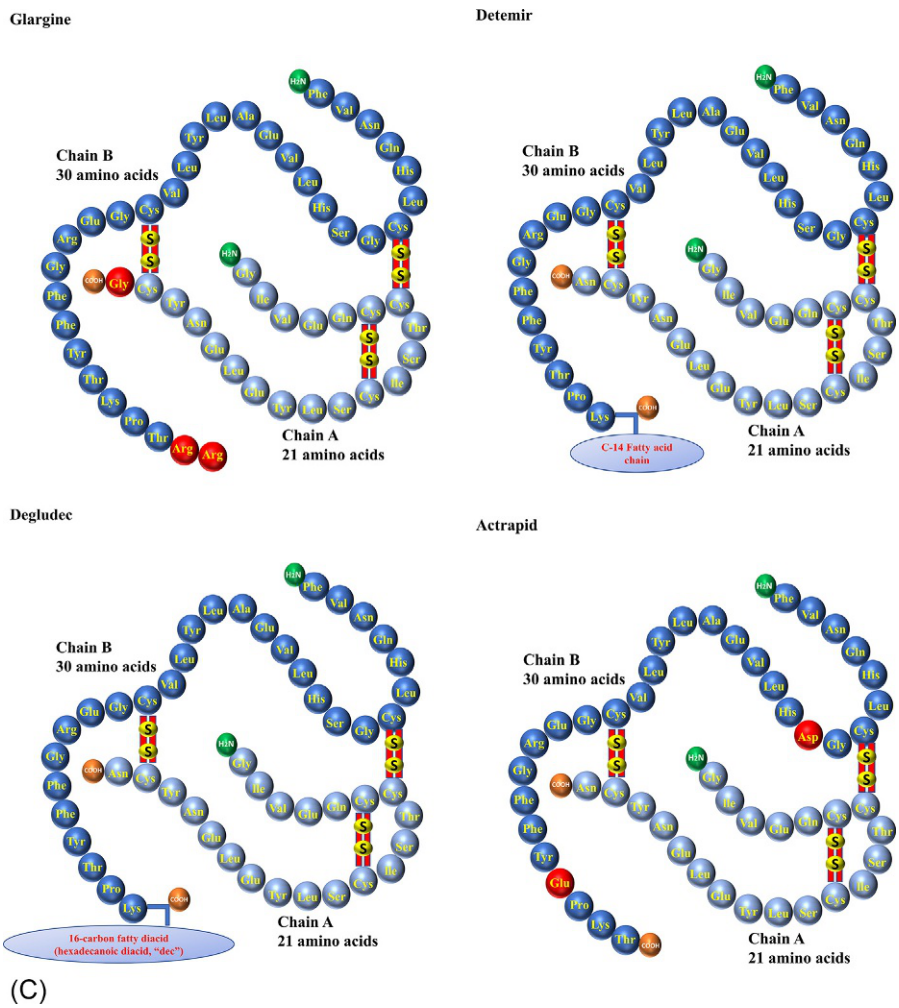

Fig. 1 cont'd (C) long-acting insulin analogs.

1930, a chemist called Hagedorn modified insulin to prolong its action by adding protamine to the insulin structure. Whereas, Scott and Fisher improved insulin structure by adding zinc. In 1978, David Goeddel and his colleagues prepared the first recombinant DNA human insulin by utilizing and combining the insulin A- and B-chains presented in *Escherichia coli* genes [22]. Then, the first insulin utilizing rDNA technology, Humulin† R (rapid) and N (NPH, intermediate-acting), were marketed in 1982. The amino acids site modifications in the insulin led to the discovery of short acting insulin such as Lispro that approved in 1996, aspart in 2000 and glulisine

in 2004. In addition, the glargine, approved in 2000 and detemir in 2005 which were basal insulin analogs in the market [16, 22, 23].

Humulin, the first recombinant human insulin synthesized by bacteria, was approved by the Food and Drugs Administration (FDA) in 1982 [24]. Through genetic engineering, insulin analogs improved control of blood glucose in the diabetic population. Modifications of the insulin backbone gave rise to new insulin analogs, as shown in Fig. 1B and C. The rapid-acting insulin analogs have a rapid onset of action, about 15–30 min following administration, and include insulin lispro [25, 26], aspart [27], and glulisine [28]. The duration of action of rapid-acting insulin analogs is around 5 h. Short-acting insulin analogs have an onset of action of about 30 min to 1 h; their duration of action is around 6–8 h with a peak of approximately 2–4 h. Short-acting insulin analogs include actrapid, hypurin, and neutral [23]., Furthermore, the intermediate-acting insulin analogs include lente and the Neutral Protamine Hagedorn (NPH) [29], have an onset of action of approximately 1–2 h and a duration of action of approximately 10–16 h [30]. Insulin detemir, glargine, and ultralente are classified as long-acting insulin, their onsets of action are around 2 h [31], with a duration of action that extends to about 36 h and the peak occurring between 6 and 20 h after administration [32] as shown in Table 1.

5. Parenteral insulin delivery

Insulin is protein that cannot be orally administered since it is degraded by gastrointestinal tract enzymes and acidic pH. SC insulin injections are used in patients with DM type I to normalize blood glucose level (BGL) when the preprandial glucose level is 70–130 mg/dL, and when postprandial blood glucose is less than 180 mg/dL. Administration of insulin to the subcutaneous fatty layer slows the absorption rate, but this route is faced with patient pain, poor compliance, and nonadherence [33]. Also, multiple daily injections may lead to local hypertrophy, infections, and lipid deposits at the injection sites, skin allergy, and resistance to insulin [34]. Therefore, the development of insulin pumps helped to overcome some SC disadvantages. Devices such as insulin pumps deliver insulin continuously via the subcutaneous route by following the basal body release in a 24-h phase. It has the advantage of providing the patient with precise insulin dosages, but its efficacy is limited by mechanical failure and inflammation at the site of attachment [35].

The CS route of insulin administration has been linked to many disadvantages such as repeated injection carries about pain, infection, and sometimes nerve damage, which causes poor patient compliance. Other disadvantages of the oral route including the gastrointestinal tract (GIT) barriers such as proteolytic enzymes and low permeability of the intestines that reduce the bioavailability of insulin [36], led to increase the need to discover new nonparenteral routes.

Table 1 Human insulin analogs available at the market.

Insulin	Brand name	Onset (h)	Peak (h)	Duration (h)
Rapid-acting insulin				
Insulin Lispro	Humalog	Within 15 min	~1	3 to 5 h
Insulin Aspart	Novolog	Within 15 min	1–3	3 to 5 h
Insulin Glulisine	Apidra	0.25–0.5	0.5–1	4 h
Technosphere insulin	Afreeza	Within 5 min	15 min	about 3 h
Short-acting insulin				
Regular Human	Humulin R Novolin R	~1	2–4	5 to 8 h
Intermediate-acting				
(Neutral Protamine Hagedorn) NPH Human	Humulin N Novolin N	1–2	4–10	14 h
Long-acting				
Insulin Detemir	Levemir	3–4	6–8	up to 20–24 h
Insulin Glargine	Lantus Basaglar Toujeo	1.5	Flat	24 h
Insulin Degludec	Tresiba	1	9	42 h

6. Nonparenteral insulin delivery

Several insulin delivery routes were used for the management of DM. The subcutaneous (SC) route is most frequently used [37], but others included nonparenteral routes such as oral, rectal, buccal, pulmonary, nasal, and intravaginal as well as parenteral such as transdermal [38, 39] have been explored. Table 2 showed a list of the clinical trials using several routes of delivery for insulin or its analogs until December 2017.

Gelfoam- based eye device has been established for ocular delivery of insulin by insertion into the lower conjunctival sac causing patient inconvenience, and till now, no clinical trial has been reported [39, 40].

Table 2 Pulmonary, buccal, oral, and nasal insulin delivery systems appearing on clinicalTrials.gov.

Pulmonary insulin delivery			
Product name	**Delivery system technology**	**Phase**	**NCT number**
Dance-501	Inhaled insulin administered using Adagio-01 inhaler device	I and II	NCT02713841
		I and II	NCT02716610
Afrezza	Technosphere® particles formed using the carrier fumaryl diketopiperazine (FDKP)	I	NCT00673621
		II	NCT00662857
		I	NCT00674050
		II	NCT00747006
		III	NCT00700622
		III	NCT00642616
		III	NCT01196104
		III	NCT01451398
		III	NCT01445951
		I	NCT01544881
		I	NCT02485327
		I	NCT02470637
		II	NCT02527265
		I and II	NCT03234491
		II	NCT03324776
		IV	NCT03143816
		IV	NCT03313960
Buccal insulin delivery			
Product name	Technology	Phase	NCT number
Oral-lyn™	Formulation contains surfactants as absorption enhancers to form insulin-containing micelles.	III	NCT00668850
Oral insulin delivery			
Product name	Technology	Phase	NCT number
GIPET® I	Tablet preparation includes micelles formed with absorption enhancers	I	NCT01809184
		I	NCT01796366
		I	NCT01334034
		I	NCT01028404
		I	NCT01931137
		I	NCT02304627
		I	NCT01597713
		II	NCT02470039
		I	NCT02479022
ORMD-0801	ORMD-0801 contains enteric-coated capsule that incorporates insulin with protease inhibitors and absorption enhancers that help delivery in small intestine	II	NCT00867594
		II	NCT01889667
		II	NCT02094534
		II	NCT02535715
		II	NCT02496000
		II	NCT02954601

Continued

Table 2 Pulmonary, buccal, oral, and nasal insulin delivery systems appearing on clinicalTrials.gov.—cont'd

Pulmonary insulin delivery			
Product name	**Delivery system technology**	**Phase**	**NCT number**
Oshadi Oral Insulin	Oshadi carrier contains mixture of silica NPs with hydrophobic surface and branched polysaccharide. Insulin embedded in oil or mixture of oils	I	NCT01120912
	Oshadi Icp (insulin, proinsulin, and C-peptide in Oshadi carrier)	I and II	NCT01772251
		II	NCT01973920
HDV Insulin	In hepatocyte-directed vesicle (HDV) insulin gel capsules, insulin is bound to HDV, which is <150 nm in diameter; phospholipid bilayer has specific hepatocyte-targeting molecules then added biotin-PE	II and III	NCT00814294
		II	NCT02794155
		II	NCT03156361
		II	NCT03096392
IN-105	Tablet formulation IN-105 oral insulin (called Tregopil) is modified form of human insulin in which free amino acid group on Lys-β29 residue is covalently bonded through a nonhydrolysable amide bond to small PEG molecule.	I	NCT01035801
		II	NCT03430856
ORA2	Insulin in dextran matrix capsule	I and II	NCT00990444
		I	NCT01114750
Nodlin	Insulin nanoparticles with bioadhesive nanoencapsulation (NOD Tech)	I	ChiCTR-TRC-12001872)
Nasal insulin delivery			
Product name	Technology	Phase	NCT number
Nasulin™	Intranasal insulin spray contains CPE-215	II	NCT00850161
		II	NCT00850096

Insulin can also be delivered intranasally, the nasal cavity has the largest absorption surface area [41] and is also highly vascularized and may provide direct protein absorption [41]. However, the route has some disadvantages, such as the rapid clearance as it requires crossing through the mucus layer [42]. Liposomes, microemulsions, and solid lipid nanoparticles have been used to deliver insulin through the intranasal route. The inhibition of proteolytic enzymes was proved beneficial in surfactants-based nasal insulin delivery [43], as well as in case of gellified insulin [44], bioadhesive microspheres [45], phospholipids [46], and chitosan nanoparticles [45]. In a study using rabbits, insulin-loaded liposomes

nasally administered led to improved glycemic control and had a 13.3% bioavailability [47, 48]. One study created multilamellar vesicle liposomes composed of l-α-dipalmitoylphosphatidylcholine (DPPC) with cholesterol (Ch), soybean-derived sterylglucoside (SG), or its sterol (SS) [48]. Therefore, liposomes reformed with enhancer are able to attack the nasal mucosa, and then the insulin within the liposomes can penetrate the nasal mucosa.

Insulin can also be delivered through the lungs using an inhaler. The lungs have huge surface area, and the alveoli are only one single highly vascularized layer. Inhaled insulin thus have shown its potential to normalize BGL [49]. A major limitation to pulmonary delivery can be related to the use of inhalers by patients, with slight verification of the inhaling technique, the dose can fluctuate remarkably [50]. Furthermore, pulmonary delivery route increases the risk of acute episodes of bronchospasm in asthmatic patients [51]. Pulmonary intake of insulin can be enhanced by liposomes [52].

In another study, poly (lactide-*co*-glycolide) (PLGA) a copolymer of poly lactic acid (PLA) and poly glycolic acid (PGA) was used to encapsulate insulin; the PLGA microcapsules (PLGA-Hp55 particles) PHNP showed a constant insulin release and an extended duration of action. The bioavailability of oral administered PHNP compared with subcutaneous injection of (1 IU/kg) dose in diabetic rats was $6.27 \pm 0.42\%$ [53]. Exubera was approved by the FDA in 2006, as the first inhaled insulin powder formulation (particle size 1–5 μm). The product was available in 1 and 3 mg and is administrated using an enhance inhaler. Pharmacokinetic and pharmacodynamic profiles of the product were similar to insulin aspart formulation, taking 10–15 min for pharmacological effects to be seen after administration [54]. There was a marked reduction in postprandial glucose level as with the use of Exubera in clinical trials with uncontrolled patients with DM type I and type II [55, 56]. The FDA later approved Afrezza in 2014 for the control of postprandial BGL. Desired effects were seen within 15 min of administration and for a duration of action of approximately 3 h [57–59]. Other inhaled insulin systems had been tested in clinical trials shown in Table 3.

Insulin inhalation has become an alternative route for insulin SC injections. But, the withdrawal of products that had already reached the market and another that were tested in clinical trials showed significant concerns about upcoming discoveries of insulin pulmonary administration.

The Exubera inhaler that reflected poor sales number was withdrawn from the market by Pfizer after a year because the device size, its high cost, and the activation of the air pump in the inhaler took seconds to minutes which consumed time. In addition, it was detected that the use of Exubera can generate antibody production against insulin in the body [61]. However, doctors were asking patients who were using Exubera to check their lung function at regular time period according to FDA-approved standards to avoid lung complications. Moreover, there were no studies about long time usage of inhaled insulin and the risk of lung cancer [61].

Table 3 Clinical trials involving inhaled insulin products [60].

Inhaled insulin system	Manufacturer	Delivery system	Phase
Exubera®	Pfizer	Fine dry-powder insulin Air-assisted mechanism disperses the powder from single-dose blisters into a respirable cloud captured in a holding chamber	Phase III Filed for approval in Europe Recommended for approval in USA
AERx® insulin Diabetes Management System (iDMS)	Aradigm and Novo Nordisk	Aqueous mist inhaler Single-use insulin strips with a handheld, breath-activated, microprocessor-controlled device	Phase III
Advanced Inhalation Research (AIR) System	Alkermes and Eli Lilly & Company	Breath-activated dry-powder inhaler	Entering phase III
Technosphere Insulin System	MannKind Corporation	Proprietary dry-powder technosphere formulation Inhaled using MedTone® inhaler	Phase III
Aerodose™	Aerogen and Disetronic Medical Systems	Liquid insulin formulation (Humalin I™ 500 units)	Phase II
Inhaled Insulin	Kos Pharmaceuticals Inc.	Dry crystals propellant inhaler	Phase II
Bio-Air	BioSante Pharmaceuticals	Coated dry particles	Preclinical

7. Use of nanoparticles for oral insulin delivery

The application of nanomedicine in protein or peptide delivery is opening new opportunities for the management of various diseases, including DM. Nanoparticles are small in size and can encapsulate peptide drugs like insulin to protect them from the adverse GI environment [62–64]. Insulin encapsulation improved pharmacokinetic parameters, bioavailability, and efficacy in the management of DM [65]. Nanoparticles for oral delivery can be prepared by several ways like microemulsion, polyelectrolyte complexation self-assembly method, ionic gelation, complex coacervation, and reverse micelle formation [66]. The absorption of nanoparticles from the GIT is dependent on the particle size,

shape, charge, and coating (surface chemistry) [67, 68]. A study by He et al. found that both enterocytes and M cells internalize rhodamine B labeled carboxylated chitosan grafted nanoparticles with a diameter of 300 nm in diameter or less more efficiently compared to bigger sized particles [69]. Wang et al. used silica nanoparticles (100–500 nm) conjugated with polyethyleneimine-coated carbon dots (PCD) to promote transepithelial absorption; the nanoparticles were also coated with polyethylene glycol (PEG) to enhance mucus permeability [70]. Natural polymers like chitosan, dextran, alginate, casein, or gelatine are the most commonly used for the oral delivery of insulin. These polysaccharides and proteins are nontoxic, hydrophilic, and biocompatible [37].

8. Chitosan-based nanoparticles

Chitosan is a nontoxic polysaccharide that adheres to the mucosal surface and disrupts the tight junctions [71]. It is a natural biodegradable polysaccharide with excellent adhesion property and biocompatibility [72]. Chitosan consists of deacetylated glucosamine and *N*-acetyl-D glucosamine [73]. Chitosan physiochemical properties depend on the pH, molecular weight (MW), and degree of deacetylation of chitin. It is positively charged on the surface and form hydrogen and ionic bonds to the negatively charged sialic-acid present in the mucin, which gives it a mucoadhesive property in the gastrointestinal tract (GIT) [74]. An oral delivery study using chitosan complexed with oleic acid and labrasol (PEG 8 caprylic/capric glycerides), a surfactant, showed a hypoglycemic effect in diabetic rats after 3 h of oral administration of a dose equivalent to insulin 50 IU/kg up to 12 h compared to subcutaneous injection [75]. Moreover, chitosan nanoparticles protected insulin from enzymatic degradation and preserved the insulin activity and stability for 1 month at room temperature 25°C. Additional studies have been done and are shown in the following Table 4.

8.1 Chitosan and poly(γ-glutamic acid)

A pH-responsive nanoparticle delivery system was assessed for the oral delivery of insulin, the nanocarrier containing chitosan (positive charge) and poly-γ-glutamic acid(γ-PGA) (negative charge) was synthesized with a simple ionic gelation method [87, 90, 97]. Sadeghi et al. proposed a polyelectrolyte complexation technique that allowed direct interaction between (−) charged insulin and (+) charged polymers [97]. Self-assembled nanoparticles with a pH-sensitive characteristic were created by mixing the anionic poly-γ-glutamic acid solution with a chitosan solution in the presence of magnesium sulfate (MgSO4) and sodium tripolyphosphate (TPP) using ionic gelation method which resulted in 50% reduction of the BGL up to 10 h following oral administration [82].

Hochman et al. created a DTPA (diethylene triamine pentaacetic acid) conjugated to γ-PGA-chitosan nanoparticle to encapsulate insulin. The insulin-loaded nanoparticles reduced the BGL by 50% in a diabetic rat model induced by streptozotocin (STZ).

Table 4 Chitosan-based insulin nanoparticles.

Insulin-loaded chitosan nanoparticles			
Nanocarrier	**Method of synthesis**	**Dose**	**In vivo observation/reference**
Chitosan	Ionic gelation	oral: 21 IU/kg + SC: 1 IU/kg	BGL reduced to 80-–120 mg/dL for more than 8 h/[76]
	Ionic gelation	oral: 10 IU/kg + SC: 1 IU/kg	BGL reduced/[77]
	Ionic gelation	oral: 50 or100 IU/kg	50 IU/kg: 52.4% BGL reduced at 19 h and 100 IU/kg: BGL reduced after 6–24 h/[78]
	Radical polymerization	oral: 100 IU/kg + SC: 1 IU/kg	10–40% BGL reduced and effect sustained up to 10 h/[79]
	Ionic gelation	Oral: 15 or 30 IU/kg + SC: 2.5 IU/kg	15 IU/kg: 20% BGL reduced at 6 h and 30 IU/k g: 60% at 6 h/[80]
	Polyelectrolyte complexation	oral: 25 IU/kg	BGL reduced after injection ascending colon/[81]
	Ionic gelation	Oral: 30 IU/kg + SC: 2.5 IU/kg	50% BGL reduced at 10 h/[82]
	Polyelectrolyte complexation	Oral: 50 IU/kg + SC: 1 IU/kg	Fasting state: BGL reduced to 40% at 8 h and nonfasting state: BGL reduced to 10% at 8 h/[75]
	Polyelectrolyte complexation	Oral: 50 IU/kg	BGL reduced to 35%/[83]
	Ionic gelation	Oral: 30 IU/kg + SC: 5 IU/kg	BGL reduced at 5 h using 10% (Eudrag it® S100) and 30% (L10055)/[84]
	Ionic gelation	Oral: 30 IU/kg + SC: 5 IU/kg	BGL reduced 50% at 10 h/[85]
	Ionic gelation	Oral: 30 IU/kg + SC: 5 IU/kg	BGL 40% reduced at 4 h/[86]
	Ionic gelation	Oral: 30 IU/kg + SC: 5 IU/kg	BGL 50% reduced at 10 h/[87]
	Polyelectrolyte complexation	Oral: 12.5 I IU/kg + SC: 1 IU/kg	35% BGL reduced at 4 h/[88]
	Ionic gelation	Oral: 50 IU/kg + SC: 5 IU/kg	28% BGL reduced at 3 h/[89]
	Ionic gelation	Oral: 30 IU/kg + SC: 5 IU/kg	50% BGL reduced at 12 h/[90]
	Ionic gelation	Oral: 50 IU/kg + SC: 5 IU/kg	50% BGL reduced at 8 h/[91]
	Complex coacervation	Oral: 50 and 100 IU/kg + SC: 5 IU/kg	50 IU/kg: 29% BGL reduced at 4 h and 100 IU/kg: 33% BGL reduced at 4 h/[92]
	Two-step way (dialysis strategy and ionic cross linking)	Oral: 50 IU/kg + SC: 5 IU/kg	48.07% BGL reduced at 3 h/[93]
	Microemulsion method	Oral: 50 IU/kg + SC: 5 IU/kg	BGL reduced to 197 mg/dL, effect up to 12 h/[94]
	Electrostatic self-assembly process	Oral: 50 IU/kg + SC: 5 IU/kg	36% BGL reduced at 4 h/[95]
	Double emulsion solvent evaporation, ultrasonication	Oral: 20 IU/kg + SC: 2 IU/kg	50% BGL reduced at 2 h/[96]

In addition, the hypoglycemic effect was sustained for 12 h after oral administration [98]. DTPA is a complex agent able to deprive Ca^{2+} and Zn^{2+} of the intestinal enzymes (protease) which can improve the delivery of insulin to the systemic circulation [82, 98]. Further, ethylene glycol-bis (β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) was used instead of DTPA to chelate Ca^{2+}. EGTA demonstrated a better protective effect against proteases than DTPA in diabetic rats and maintained BGL up to 50% for a long period [90]. Moreover, these nanoparticles were packed in a gelatin capsule and coated with Eudragit S100 or L100–55 [84]. Eudragit S100 and Eudragit L100–55-coated capsules filled with nanoparticles were reported to dissolve at a pH above 7 observed in jejunum and ileum while the duodenum is more acidic with a pH of 6.6 [84].

8.2 Carboxylated, trimethylated (TMC), dimethylethyl (DMEC), carboxymethyl (CMCS), and thiolated chitosan

Generally, Chitosan is insoluble in a neutral and alkaline environment; it forms salts with inorganic and organic acids such as glutamic acid and hydrochloric acid. Thanou et al. showed that 60% of insulin is released at lower pH (pH 2) because of the pepsin penetration through the chitosan surface of loaded insulin nanoparticles in an acidic environment [99]. While, in an alkaline environment, chitosan is insoluble in the water and lose the mucoadhesive effect, which limits its use as an absorption enhancer in the duodenum (pH 6.6) [100]. To improve these limitations chitosan derivatives, including carboxylated, TMC(trimethylated chitosan), DMEC(dimethylethylchitosan), CMCS(carboxymethyl chitosan), and thiolated chitosan, have been introduced to formulate nanoparticles with enhanced oral bioavailability and BGL lowering effect [95, 99, 101]. TMC amine group can be protonated to improve the water solubility of chitosan in an alkaline environment, which facilitates the insulin penetration. Therefore one study assessed the stability of TMC-γ-PGA nanoparticles compared to chitosan-γ-PGA and demonstrated that only 30% of the encapsulated insulin was released at pH 7.0 [99].

As mentioned before, orally administered NPs require opposing surface properties to permeate the mucus layer and is transported through the GIT epithelial cells. Several methods have been developed to solve the physical, chemical, and enzymatic barriers (Fig. 2) [102]. One method formulated nanoparticles covered with poly(ethylene glycol) (PEG) to create an hydrophilic surface with neutral charge to enhance the penetration through the mucus layer [103, 104]. Another strategy was to target goblet cells that are responsible for mucus production with a high-affinity peptide, CSKSSDYQC (CSK) [105]. A modified formulation of TMC conjugated with CSK resulted in the accumulation of its content within the goblet cells. The formulation increased the uptake of the nanoparticle by the microvilli and enhanced the absorption of insulin through clathrin and caveolae endocytosis [89].

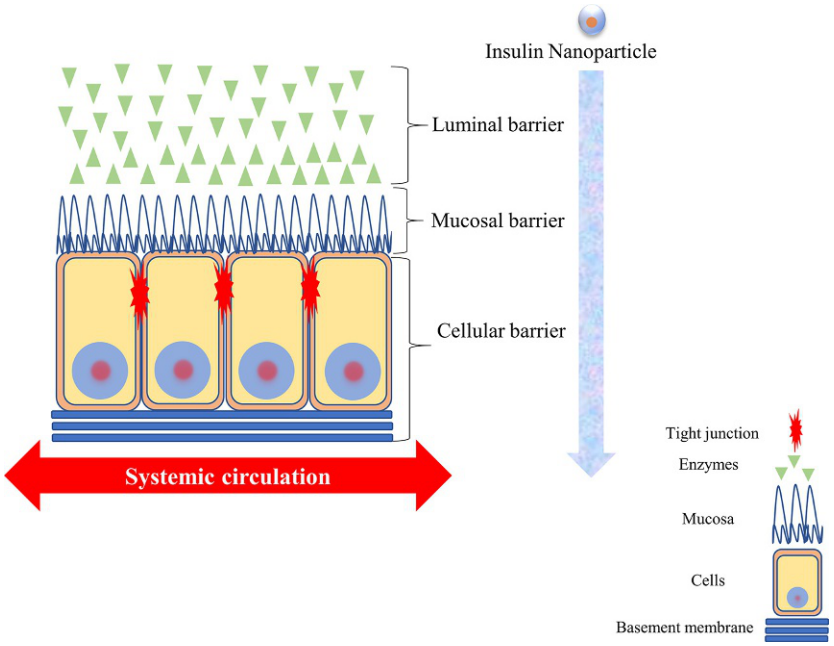

Fig. 2 Possible barriers to oral insulin delivery nanoparticles.

8.3 Vitamin B12-conjugated chitosan

The development of multilayered nanocarriers based on a layer-by-layer (LBL) synthesis has been used to formulate insulin nanoparticle and showed promising drug-release efficacy results, bioavailability, and stability at gastric pH [106, 107]. Francis et al. used vitamin B12 as a transport system to increase GIT absorption. This study examined the oral delivery of insulin by LBL technique (LBL can be prepared by alternate and repeated deposition of a polyelectrolyte on a solid surface via electrostatic interactions) with vitamin B12 (VitB12) conjugated to chitosan and alginate to form VitB12-Chi-CPNP [94]. This nanocomplex binds to intrinsic factor (IF) called cubulin, which is secreted by the gastrointestinal mucosa in the ileum and undertaken by receptor-mediated endocytosis [108]. The VitB12-Chi-CPNP formulation increased the insulin transportation across a Caco-2 cell monolayer in vitro by 14% as a result of the uptake of vitamin B12 by endocytosis and paracellular absorption in comparison to unmodified chitosan nanoparticles. Even though the BGL reduction was delayed by 1–2 h, the VitB12-Chi-CPNP complex was capable of lowering the BGL up to 12 h [109].

8.4 Chitosan and poly(ethylene glycol) (PEG)ylation

The combination of chitosan and PEG plays an important role in enhancing the penetration of nanoparticles through the mucus layer [110]. De Sousa et al. demonstrated the

effect of a combination of chondroitin sulfate and PEG with chitosan on intestinal retention. The result showed a 3.7-fold better retention capacity in the intestinal mucosa compared to unmodified nanoparticles; for the first 4 h, there was 65% of insulin released from the nanocomplex into simulated intestinal fluid, giving a mucus clearance time about 4–6 h, a time sufficient to overcome the mucus barrier [111].

9. Alginate-based nanoparticles

Alginate is a hydrophilic, biodegradable, and nontoxic anionic polysaccharide isolated from marine brown algae. It is composed of blocks of (1–4)-linked β-D-mannuronic acid and α-L-guluronic acid residues [112]. The guluronic acids cross-linked with Ca^{2+} ions create a gel-like matrix, allowing insulin retention [113]. Therefore, it can form polyelectrolyte complexes with polycations. An ionotropic pre-gelation of alginate with calcium chloride ($CaCl_2$) and complexations between chitosan and alginate have led to the formation of nanoparticles [113]. Sarmento et al. have shown that these nanoparticles led to the reduction of BGL by 40%, in diabetic rats dosed with the insulin equivalent of 50 IU/kg and 100 IU/kg; the hypoglycemic effect was sustained for up to 18 h [114]. Recently, a cationic β-cyclodextrin polymer (CPβ-CDs) was added to enhance the solubility and stability of the insulin alginate-chitosan nanocarrier. However, the amount of insulin released was not suitable in this formulation, about 40% released within 2 h in simulated gastric fluid (SGF) [115]. Additional studies using alginate as a nanocarrier to deliver insulin are shown in Table 5.

10. Dextran-based nanoparticles

Dextran sulfate is a natural, nontoxic, hydrophilic, biodegradable, and negatively charged polysaccharide that can be cross-linked with the positively charged insulin. Its chemical property offers protection against proteases. The conjugation with vitamin B12 allows the complex to escape gastric proteolytic enzymes in the GIT [117]. According to a study done by Chalasani et al., a VitB12 nanoparticle conjugated system led to the

Table 5 Alginate-based insulin nanoparticles.

Insulin-loaded alginate nanoparticles			
Nanocarrier	**Method of synthesis**	**Dose IU/Kg**	**In vivo observation/reference**
Alginate	Ionotropic pregelation and polyelectrolyte complexation Complex coacervation	Oral: 50 and 100 IU/kg +SC: 2.5 IU/kg oral: 10 IU/kg +SC: 1 IU/kg	59% BGL reduction in 50 IU/kg and 55% BGL reduction in 100 IU/kg/[114] 47% BGL reduced at 5 h/[116]

improvement of the oral delivery of insulin. The treatment with insulin-VitB12 dextran conjugated NPs (20 IU/kg) led to a significant lowering of BLG for at least 24 h [118].

Sarmento et al. showed that BGL was reduced by 22% and 60% after oral administration of 50 and 100 IU/kg equivalent doses of encapsulated insulin in diabetic rats, respectively; the BGL was stable up to 24 h [119]. Tiyaboonchai et al. used insulin encapsulated into dextran sulfate-chitosan nanoparticles, the oral administration of equivalent doses of insulin at 50 and 100 IU/kg lowered the 40% BGL at 4 h [120] as shown in Table 6.

Also, multilayered nanoparticles consisted of alginate, dextran sulfate, chitosan, and albumin that were capable of reducing the BGL and maintaining insulin bioavailability

Table 6 Dextran-based insulin nanoparticles.

Insulin-loaded dextran nanoparticles			
Nanocarrier	**Method of synthesis**	**Dose**	**In vivo observation/reference**
Dextran	–	SC: 2.5 IU/kg	40% BGL reduced at 4 h/[120]
	Ionotropic pregelation	Oral: 50 or 100 IU/kg + SC: 2.5 IU/kg	50 IU/kg: 22% BGL reduced; 100 IU/kg: 60% BGL reduced [119]
	Nanoemulsion dispersion/in situ triggered gelation	SC: 1 or 4 IU/kg	SC:1 IU/kg: 76% BGL reduction within 4 h; SC: 4 IU/kg: 89% BGL reduced/[64]
	Emulsion	Oral: 20 IU/kg + SC: 2.5 IU/kg	70–75% BGL reduced at 5 h/[121]
	Emulsion	Oral: 10 and 20 IU/kg + SC: 0.4 IU/kg	70–75% BGL reduction/[118]
	Nanoemulsion dispersion/in situ triggered instantaneous particle gelation	Oral: 50 IU/kg + SC: 0.4 IU/kg	52% BGL reduced at 12 h/[122]
	Emulsification/internal gelation technique, polyelectrolyte complexation	Oral: 50 IU/kg + SC: 5 IU/kg	40% BGL reduction/[123]
	Emulsification/internal gelation technique	Oral: 50 IU/kg + SC: 2 IU/kg	43% BGL reduction/[124]
	Emulsification/internal gelation technique, polyelectrolyte complexation	Oral: 50 and 10 0 IU/kg + SC: 2 IU/kg	45% BGL reduced at 8 h; Effect continued up to 12 h/[125]

over 24 h [123]. These multiple coatings increased insulin absorption by clathrin-mediated endocytosis [126]. Moreover, intraperitoneal glucose tolerance tests were done to examine the biological activity of the insulin provided by the multilayered nanoparticles in both DM rat models. The BGL was reduced in diabetic rats after glucose challenge [125]. Additionally, the use of VitB12-dextran conjugated nanoparticles increased insulin absorption by targeting the intrinsic factor (IF) receptor. In comparison to other nanoparticle delivery systems, BGL was reduced by 75% and prolonged hypoglycemia for 54 h in diabetic rats at a lower equivalent dose of insulin (20 IU/kg) [118]. Also, a ternary inter-polyelectrolyte complex of insulin, dextran sulfate, and poly (methylaminophosphazene) (PMAP) was only studied in vitro, it fully protected insulin from gastric enzymes [127].

11. Polymer-based nanoparticles

Synthetic polymers like poly (ε-caprolactone) (PCL), polylactic acid (PLA), and poly(lactic-*co*-glycolic acid) (PLGA) have been used in oral insulin delivery systems because of their biodegradability and biocompatibility properties as shown in Tables 7–10.

11.1 PLGA

PLGA is a biodegradable synthetic polymer that has been used for oral insulin delivery. Insulin was encapsulated into PLGA nanoparticles using a reverse polymeric micelle-solvent evaporation system to improve insulin liposolubility and entrapment efficiency up to 90% in the hydrophobic matrixes z. In this study, PLGA (2%) was solubilized in an organic solvent (dichloromethane or ethyl acetate) and then added to the insulin-phosphatidylcholine solution. One study used sodium deoxycholate with insulin, followed by an emulsion solvent diffusion method to encapsulate insulin into PLGA nanoparticles, which improved the entrapment efficiency by 93.6% and reduced BGL by 43% in diabetic rats [132]. Sun et al. showed that PLGA nanoparticles complexed with insulin prepared as hydrophobic ion pairing (HIP) method caused a rapid fall in the BGL after 12 h and lasted for 24 h [131]. The PLGA nanoparticles passed through the gastrointestinal tract and were absorbed by the M cells in the Peyer's patches [134].

Recently, a new polymer lipid hybrid nanoparticle, composed of a core PLGA and a PEG shell with an amphiphilic soybean phosphatidylcholine (SPC) intermediate layer was designed using a spray freeze-drying technique, then coated with hard gelatin and hydroxypropyl methylcellulose phthalate (HPMCP-55). The new formulated nanoparticles induced a hypoglycemic effect for 24 h and maintained insulin efficiency for 3 months when evaluated in diabetic rats [149].

Cui et al. added hydroxypropyl methylcellulose phthalate (HPMCP-55) to PLGA nanoparticles (PLGA-Hp55) to increase oral insulin delivery [53]. When comparing

Table 7 PLGA-based insulin nanoparticles.

Insulin-loaded PLGA nanoparticles			
Nanocarrier	**Method of synthesis**	**Dose**	**In vivo observation/reference**
PLGA	Nanoprecipitation solvent displacement	Intraileally: 10 or 20 IU/kg	10 IU/kg: 20% BGL reduced at 30 min; 20 IU/kg: 40% BGL reduced at 1 h/[128]
	Reverse micelle solvent evaporation	Oral: 20 IU/kg + SC: 1 IU/kg	57.4% BGL reduced within 8 h/[129]
	Modified emulsion solvent diffusion	Oral: 20 IU/kg + SC: 1 IU/kg	60% BGL reduced within 16 h/[53]
	Hydrophobic ion pairing modified (w/o/w) double emulsion	Oral: 20 IU/kg + SC: 1 IU/kg	41% BGL reduced within 4 h, and recovered to 88.9% within 16 h/[130]
	Hydrophobic ion pairing and emulsion solvent diffusion	Oral: 20 IU/kg + SC: 1 IU/kg	23.85% BGL reduced at 12 h/[131]
	Hydrophobic ion pairing and emulsion solvent diffusion	Oral: 20 IU/kg + SC: 1 IU/kg	43% BGL reduced/[132]
	w/o/w solvent evaporation technique	Oral: 15 IU/kg + SC: 1 IU/kg	45.7% BGL reduced at 8 h/[133]
	Double emulsion solvent evaporation	Oral: 50 IU/kg + SC: 5 IU/kg	14% BGL reduced after 4 h; 57% BGL reduced after 18 h/[134]
	Double emulsion solvent evaporation	Oral: 20 IU/kg + SC: 2.5 IU/kg	BGL reduced from 260 to 196.33 mg/dL after 3 h; Post-feed: BGL reduced from 203 to 97.55 mg/dL after 7 h/[135]
	Multiple solvent evaporation via ultrasonic emulsification	Oral: 50 IU/kg + SC: 5 IU/kg	32.9% BGL reduction at 10 h/[136]
	Double emulsion and solvent evaporation process, coupling reaction	Oral: 10 IU/kg + SC: 11 IU/kg	40% BGL reduced at 5 h/[137]
	Modified surface functionalization method	Oral: 10 IU/kg + SC: 11 IU/kg	BGL reduced after 3 h post-oral administration/[138]
	Double emulsion solvent evaporation	Oral: 20 IU/kg + SC: 2 IU/kg	70% BGL reduced at 7 h/[139]
	Double emulsion solvent evaporation, carbodiimide coupling process	Oral: 20 IU/kg + SC: 20 IU/kg	BGL reduced within 4 h/[140]
	Single-step nanoprecipitation	Oral: 50 IU/kg + SC: 2 IU/kg	40% BGL reduced at 4 h/[141]

Table 8 PLA-based insulin nanoparticles.

Insulin-loaded PLA nanoparticles			
Nanocarrier	**Method of synthesis**	**Dose**	**In vivo observation/reference**
PLA	Nanoprecipitation	Oral: 1.1 IU/kg + SC: 3.3 IU/kg	BGL reduced for at least 15 h/[142]
	Nanoprecipitation	Oral: 50 IU/kg + SC: 2 IU/kg	40% BGL reduced for at least 4 h/[143]

Table 9 PAA-based insulin nanoparticles.

Insulin-loaded PAA nanoparticles		
Nanocarrier	**Components**	**In vivo observation/ reference**
PAA	PAA (15 kDa) + palmitic acid-N hydroxysuccin imide ester + quaternary ammonium moieties	only in vitro/[144]
	PAA (15 kDa) + cholesteryl chloroformate	only in vitro/[145]
	PAA (15 kDa) + palmitic acid-N hydroxysuccin imide ester + quaternary ammonium moieties	only in vitro/[146]

Table 10 CPP-based insulin nanoparticles.

Insulin-loaded nanoparticles containing CPP			
Nanocarrier	**Method of synthesis**	**Dose**	**In vivo observation/ reference**
CPP	Self-assembly in the form of polyelectrolyte complex		60% BGL reduced/[147]
	Two-step approach based on self-assembly, radical polymerization	Oral: 75 IU/kg + SC: 5 IU/kg	50% BGL reduced/[148]

PLGA-Hp55 to PLGA nanoparticles, the insulin released was 19.77% and 50.46%, respectively, in simulated gastric fluid over 1 h. In vivo study demonstrated that the insulin PLGA-Hp55 nanoparticles lowered the BGL by 60% [53]. Moreover, PLGA/Eudragit RS nanoparticle system coated with HPMCP-55 have also been used for the oral insulin delivery and to improve the protection of insulin from proteolytic enzymes [136]. However, to overcome the mucus barrier both neutral and hydrophilic nanoparticles are generally required. The coating of PLGA nanoparticles with TMC showed

effective penetration of insulin nanoparticles into the mucus layer produced by HT29-MTX cells, a mucus-producing human colorectal adenocarcinoma cell line. Sheng et al. concluded that TMC-PLGA nanoparticle was absorbed in the GIT by both transcellular (clathrin endocytosis) and paracellular pathway (tight junction opening), and lower the BGL by 70% in diabetic rats 7 h following oral administration [139].

Moreover, Jain et al. conjugated folate (FA) to PEGylated PLGA to entrap insulin. The insulin-FA-PEG-PLGA nanoparticles enhanced GI insulin uptake through M cells and folate receptors [134]. A dose equivalent to 50 IU/kg of insulin showed a 2-fold oral bioavailability increase and no hypoglycemic shock when compared to subcutaneous injections. Insulin conjugated FA-PEG-PLGA NPs sustained the BGL for 24 h [134].

Different cell-penetrating peptides (CPP) have been conjugated to PLGA to facilitate absorption of insulin such as D-arginine octamer, L-penetratin, L-pVEC, and L-RRL helix. These CPPs have been optimized to expand insulin stability, absorption, and hypoglycemic effect [150].

11.2 Poly(lactic acid) (PLA)-based nanoparticles

PLA has similar characteristics as to PLGA, but is hydrophobic, and degrades slowly [151]. PLA was further used for oral drug delivery. PLA-b-pluronic-b-PLA (PLA-F127-PLA) vesicles were used as an insulin nanocarrier and showed hypoglycemic effect for 18.5 h [152]. Then, the PLA-F127-PLA vesicles were modified by attaching IgG-Fc fragments to target the neonatal Fc receptor (FcRn) which beneficially lower BGL using minimal equivalent dose of insulin (1.1 IU/kg) [142]. Another PLA nanoparticle coated with 40% dilauroyl phosphatidylcholine zwitterions achieved the best hypoglycemic effect with 40% BGL reduction for 4 h [143].

11.3 Polyallylamine (PAA)-based nanoparticles

The amphiphilic nanoparticles, consisting of polyallylamine (PAA) were developed to protect insulin against GIT enzymes such as trypsin and pepsin and showed a high entrapment efficiency. The in vitro results showed limited degradation of insulin by both pepsin and trypsin but no protection against α-chymotrypsin activity [144].

11.4 Polymeric nanoparticles containing CPP

Penetratin, is a CPP, which enhanced the delivery of insulin through both energy-dependent endocytosis and energy-independent transduction across the epithelial layer [147]. Recently a study demonstrated the benefice of penetratin grafting and free penetratin, to improve insulin absorption [153]. The nanoparticles that composed of penetratin and hydrophilic pHPMA (poly(N-(2-hydroxypropyl) methacrylamide) coating, exhibited excellent permeation of the mucus layer and increased epithelial uptake by

20-fold higher when compared to free insulin. The neutral properties of pHPMA facilitated the insertion of the insulin into mucus layer without being trapped. Interestingly, the oral administration of insulin, penetratin, and hydrophilic pHPMA nanoparticles lowered the BGL by 20% in diabetic rats [142]. A recent animal study showed that 50% BGL reduction occurs following the oral administration of insulin, penetratin, and pHPMA nanoparticle (dose equivalent to 75 IU/kg) [148].

12. Inorganic nanoparticles

Inorganic molecules such as gold [154], selenium [155], iron oxide [156], calcium phosphate [94], cerium oxide [157], and zinc which also has an insulin-mimetic activity have been investigated [158] for oral insulin delivery as shown in Table 11. Oral and intranasal administration of insulin-loaded gold nanoparticles reduced BGL in diabetic rats [159]. Gold-chitosan nanoparticles were biocompatible, nontoxic, and reduced BGL in diabetic rats via both oral and nasal administration, with an insulin equivalent dose of 50 and 10 IU/kg, respectively. Results of 50 IU/kg dose for oral route showed a 30.41% reduction of BGL following oral administration, and 10% through the nasal route [160]. Moreover, silica nanoparticles have also been used for oral insulin administration. Zhao et al. developed insulin-loaded nanocomplex via poring insulin in the pores of silica-coated with HPMCP-55 [161]. This study showed a high adsorption capacity and significant hypoglycemic effect after 1 h following the oral administration of insulin equivalent to 30 IU/kg orally administration [161].

13. Nanoparticles containing Eudragit

Eudragit is the manufactural name for a diverse range of polymethacrylate-based copolymers. It includes anionic, cationic, and neutral copolymers based on copolymers derived from esters of acrylic and methacrylic acid [168]. Many nanoparticles containing Eudragit have been used for oral insulin delivery as shown in Table 11. Therefore, the preparation of insulin-loaded nanoparticles has been done by a blend of Eudragit RS (polymer containing methacrylic acid esters and a small portion of trimethylaminoethyl methacrylate chloride) and biodegradable polyester (poly(-ε-caprolactone) (PCL) [162, 163]. Both Eudragit and PCL have a cationic charge which can enhance the mucoadhesive properties in the GIT and uptake by the M cells in the ileum, improving the glycemic response with a dose of insulin equivalent to 100 IU/kg [162]. Furthermore, the same nanocarrier loaded with aspart displayed a BGL reduction using a dose equivalent to 50 IU/kg. Also, aspart was protected against enzymatic degradation in GIT [163].

Several factors, such as the ratio of polymers, the volume of surfactants, and the pH of organic solvents, may affect the function of nanoparticles [169]. Other nanospheres have also received more attention for the delivery of insulin such as carbon nanospheres, which

Table 11 inorganic-based insulin nanoparticles and insulin-loaded nanoparticles containing Eudragit®.

Insulin-loaded inorganic nanoparticles and insulin-loaded nanoparticles containing Eudragit®			
Nanocarrier	**Component**	**Dose**	**In vivo observation/reference**
Gold	Gold	Oral: 50 IU/kg Nasal: 20 IU/kg SC: 5 IU/kg	Oral: 31% BGL reduction; maximal hypoglycemic effect at 3 h and Nasal: 50% BGL reduction/[159]
	Gold nanoparticle reduced by 90% deacetylated chitosan (MW 45 kDa)	Oral: 50 IU/kg Nasal: 10 IU/kg	Oral: 30.41% BGL reduction and Nasal: 10% BGL reduction/[160]
	Gold +0.5% chondroitin sulfate	Oral: 50 IU/kg	32.1% BGL reduction/[152]
Silica	Silica (TYS600F) + HPMCP55	Oral: 30 IU/kg SC: 1 IU/kg	BGL reduced to 2.67–4.85 mmol/L at 3 h
	Silica + coated with PMV [Poly(meth acrylic acid covenyl triethoxy silane) + Pluronic P123 +	Oral: 15 IU/kg + SC: 1 IU/kg	Rat: 10% BGL reduced at 2 h and 60% reduction for next 6 h till 16 h/[161]
Eudragit®	Eudragit ® RS (MW 150,000) + PCL (MW 4200) + PVA	Oral 100 IU/kg	41% BGL reduced after 4 h/[162]
	Eudragit RS® (MW 150,000) + PCL (MW 4200) + PVA	Oral: 50 IU/kg SC: 10 IU/kg	29% BGL reduction after 30 min and maximum reduction between 6 and 8 h (53%)/[163]
	Eudragit ® RL or RS + PEG 300 or glycofurol + PVA + poloxamer	Oral: 50 IU/kg SC: 1 IU/kg	Eudragit® RS—glycofurol: 25% BGL reduction at 2 h; Eudragit® RL—PEG: 5% BGL reduction at 6 h/[164]
	Eudragit® L100-coated capsule + Saccharom yces cerevisiae + chitosan solution	Oral: 50 IU/kg	33% BGL reduction at 4 h/[165]
	95% deacetylate d chitosan (100 kDa) coated with + Eudragit ® S100 + Tat + TPP	Colon injection: 20 IU/kg (rat) Oral: 10 IU/kg (mini pig)	Rat: 66.06% BGL reduced & Minipig: 40% BGL reduction at 10 h/[166]
	Polyethyle ne imine (MW 750 kDa) + dextran sulfate (MW > 500 kDa) + zinc sulfate + HPMCP55 + Eudragit ® NE 30 D + sodium glycocholate + METHOC EL™	Oral: 1.33 mg/kg SC: 0.77 IU/kg	50% BGL reduction at 6 h/[167]

can effectively transport drugs across the GIT. Amphiphilic carbon nanospheres coated with chitosan and Eudragit L-100 were able to protect insulin against enzymatic degradation and decrease the BGL by 33% after 4 h following oral administration in diabetic rats [165]. On the other hand, the alteration of Eudragit L-100 with cysteine to form a thiomer was shown to control the insulin released in an alkaline environment, improved mucoadhesiveness of nanoparticles through mucus layer and eased the insulin transportation across GIT [170].

14. Solid lipid nanoparticles

Solid lipid nanoparticles (SLNs) are colloidal particles with a diameter between 50 and 1000 nm and are characterized by a lipid-based hydrophobic delivery system that protects insulin against gastric enzymes and helps GIT absorption due to its mucoadhesive effect. In general, the SLNs can be absorbed by the intestine due to the hydrophobic surface, but the entrapment efficiency of hydrophilic insulin within the SLNs is low. The cetyl palmitate-based SLNs were coated with poloxamer 407 to protect the nanoparticles against aggregation in simulated gastric fluid (SGF) and reduced the BGL by 20% over 24 h [171]. Another study used chitosan-coated SLNs containing Witepsol 85E, which is a hard fat with a melting range above body temperature and is used to improve the paracellular transport across Caco-2-HT29 joined culture by two to threefold higher [172]. Another study used SLNs modified with lectin and wheat germ agglutinin (WGA), but the relative oral bioavailability was low and required the combination with protease inhibitors and the improvement of the drug entrapment efficiency [173]. One approach used METHOCEL Cellulose Ethers and PLGA polymers to augment the stability during the preparation, and improve insulin-loading efficiency [174]. Other approach used PEG to preserve the bioactivity of insulin moiety in the GIT and increase the entrapment efficiency [175]. These different studies are shown in Table 12.

Besides, a novel approach of reverse micelle double emulsion method using sodium cholate and SPC was employed to formulate nanoparticles [180]. One study reported the loading of insulin in the SLN nanoparticles, with an efficiency of 94% [179]. Moreover, SLNs were modified by CPP to improve the oral delivery of insulin to provide a relative oral bioavailability of 10.4% [176].

15. Nanomedicine formulations in clinical trials

Pharmaceutical laboratories have developed various nano-formulations in the last few years to improve the delivery of insulin. The main hindrances in the transition of these formulations to clinical use are the biocompatibility issues, low oral bioavailability, and significant interindividual variations.

Table 12 solid lipid nanoparticles-based insulin nanoparticles.

Insulin-loaded solid lipid nanoparticles			
Nanocarrier	**Method of synthesis**	**Dose**	**In vivo observation/reference**
SLN	Modified double dispersion	Oral: 50 IU/kg SC: 2 IU/kg	40% BGL reduced at 3 h/ [173]
	Modified solvent emulsion evaporation (w/o/w double emulsion)	Oral: 50 IU/kg SC: 2.5 IU/kg	20% BGL reduced after 6hr/[171]
	Spontaneous emulsion solvent diffusion	Oral: 20 IU/kg SC: 1 IU/kg	11% BGL reduced at 0.25 h and 36% reduced at 3 h/[176]
	Modified solvent emulsification-evaporation (w/o/w double emulsion)	Oral: 25 IU/kg SC: 2.5 IU/kg	23.8% BGL reduced at 12 h/[172]
	Spontaneous emulsion solvent diffusion	Oral: 25 IU/kg SC: 2 IU/kg	73.2% BGL reduced at 2 h/[177]
	Ionic gelation	In situ into ileal segments: 25 OR 50 IU/kg	25 IU/kg: 45% BGL reduced at 1 h and 50 IU/kg: 57.18% BGL reduced at 1 h/[178]
	Modified solvent injection	Oral: 40 and 60 IU/kg SC: 2 IU/kg	40 IU/kg: 47% BGL reduced at 4 h and 60 IU/kg: 65% BGL reduced/[179]
	Emulsification solvent evaporation technique	Oral: 50 IU/kg + SC: 2 IU/kg	50% BGL reduction/[175]

The nasal route is considered as a promising route for delivery of nanomedicine due to the high vascularity and the large surface area [86]. But, most insulin nanoformulations failed to enhance the uptake of insulin in clinical trials [181]. Another promising route of insulin delivery is the pulmonary route since it highly vascularized, and the monolayer alveoli epithelium allows for rapid drug absorption. The use of Exubera dry powder formulation by Novo Nordisk who relied on this technology failed and withdrawn. The administration of insulin-calcium phosphate and PEG to the respiratory tract has been seen to improve the deposition of insulin in rats [10]. One example of these formulations is Ora-Lyn commercialized by the Generex Biotechnology. It is an insulin spray that is currently under Phase III clinical trial in the USA, Canada, and Europe. Ecuador and Lebanon have already been approved for Ora-Lyn inhaler use in the diabetics [182]. Ora-Lyn inhaler was approved by the FDA as investigational new drug to be used in type I and type II DM patients with life-threatening conditions. The formulation was created to deliver insulin through the buccal mucosa, as a way of bypassing the intestinal and liver's first-pass effects. Ora-Lyn consists of an insulin-loaded micelle and a surfactant

to improve absorption properties. The Oral-Lyn formulation uses the buccal delivery route. The buccal mucosa is easily accessible and has a large surface area, but the formulation must have absorption enhancers like sodium lauryl sulfate and surfactants to increase membrane permeability [183]. The vaginal and rectal delivery options have the advantage of bypassing first-pass metabolism. Surfactants and absorption promoters can be added to the insulin nanoparticles to achieve the highest hypoglycemic effect [184].

The oral route for delivery of insulin nano-formulations has been considered the safest and convenient method of delivering insulin to the liver [185]. However, the stomach is highly acidic, and together with the presence of proteolytic enzymes, there can be structural instability of insulin protein but with using nanocarriers, insulin can be more stable at acidic environment [186]. The nanomedicines developed for oral delivery and assessed in clinical trials were formulated to withstand the acidic nature of the gastric contents [187]. Most insulin nano-formulations developed for oral delivery are still in clinical trial phases I or II. IN-105, a formulation created by Biocon, is in clinical trial phase II. The modified insulin was conjugated through a spacer to a PEG molecule to improve insulin delivery. Diasome Pharmaceutical Company developed an insulin-loaded liposome harboring a biotin-phosphatidylethanolamine on its surface to target the liver. The insulin-loaded hepatocyte directed vesicle (HDV-I) is stable in the upper gastrointestinal tract and bloodstream. Each capsule has a low amount of insulin, and the targeting strategy to the hepatocytes was meant to decrease the risk of insulin overdose [188]. The HDV-I has undergone through several phase I and II clinical trials [189, 190]. Nodlin formulation by NOD Pharmaceuticals is an insulin nanoparticle with bioadhesive embedded in enteric-coated nano capsules. This formulation underwent a phase I clinical trial [191]. Another formulation Macrulin is currently in phase II clinical trial in Europe. The formulation is a lecithin-based microemulsion developed to increase the bioavailability of insulin in the circulation [192, 193]. Also, the Jordanian Pharmaceutical Manufacturing company has developed an oral insulin delivery system. The formulation is an insulin-loaded chitosan nanoparticle dispersed in an oily vehicle. In a phase I clinical trial, this formulation showed promising results in terms of bioavailability of insulin [75, 194]. The utilization of Merrion's GIPET Technology by the Novo Nordisk Company has led to the development of several insulin formulations using a lipid mixture with a surfactant in enteric-coated gel capsules. Most of the formulations from the Novo Nordisk Company (NN1952, NN1953, NN1954, and NN1956) have undergone phase I clinical trials for patients with type II DM [195]. Icp-insulin and proinsulin formulations have been developed by the Oshadi Drug Administration Company and assessed in phases I and II clinical trials. In these formulations, insulin is delivered by a C-peptide. The carrier in these formulations is a pharmacologically inert nanoparticles which have a hydrophobic side and polysaccharide branches [188].

In this era, where the focus is shifting to nanomedicine in the management of diabetes, there are still other alternative insulin delivery systems in development such as the use of a painless microneedle array patch for the glucose-responsive closed-loop delivery unit [196]. The patch has insulin encapsulated vesicles that are sensitive to hypoxia and hydrogen peroxide [197]. In another delivery system developed by Wang et al., glucosamine and insulin have been conjugated to promote binding to glucose transporter on RBCs cell membrane, this is a reversible event where insulin is released when there is a high BGL [198].

16. Conclusion

The subcutaneous route overwhelmingly used for insulin delivery in the management of DM is faced with several setbacks that compromised the efficient management of the disease and the compliance of patients. Over the last decades, nanotechnologies have contributed to the development of nanocarriers to improve insulin stability and targeted delivery. More recently, progress has been made to create nanocarriers compatible with the oral or nasal routes and are currently assessed in preclinical and clinical studies. As the number of DM cases is rapidly increasing worldwide in populations due to increasingly sedentary lifestyles, obese, and young, nanotechnology will have to become more personalized to achieve a therapeutic outcome and improve patient compliance.

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CHAPTER 18

Postoperative local administration of nanomedicine

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1. Introduction

In the past decades, nanotechnologies have transformed medicine and paved the way for the improvement in the diagnosis, treatments, and management of several pathological conditions such as cancer, pain, inflammation, infection, and neurodegenerative diseases [1]. More than 50 nanomedicines have been approved by the US Food and Drug Administration (FDA) in the past 25 years [2]. Organic, inorganic, and polymeric nanomedicines, including liposomes, micelles, and dendrimers are the most frequently designed nanostructures [3, 4]. The majority of the nanomedicines approved on the market are intended for parenteral administration to bypass the degradation of the drug in the gastrointestinal tract and the hepatic first-pass metabolism (Fig. 1) [3]. The parenteral routes provide the highest bioavailability and are favored for their acute and rapid effect but usually require hospital settings and are often associated with tissue damage and pain leading to poor patient compliance [5]. Nonparenteral routes, such as oral, topical, pulmonary, nasal, ophthalmic, and vaginal, have generated increasing interest with the development of new polymers and new nanoformulations. The nonparenteral routes are more suitable for sustained and chronic drug delivery and to achieve a localized or systemic therapeutic effect. The size, shape, surface coating, and bioconjugates of the nanomedicines have been implemented to address the specificity of the nonparenteral routes [6–9]. In this chapter, we will discuss the postoperative management of pain and infection by nanomedicines and nanoformulations through nonparenteral routes to achieve superior clinical outcomes while decreasing the risk of local or systemic toxicity.

2. Postoperative pain

2.1 Background

Despite the progress made into the understanding of the mechanism of pain, most patients who experienced surgical procedures coped with acute postoperative pain,

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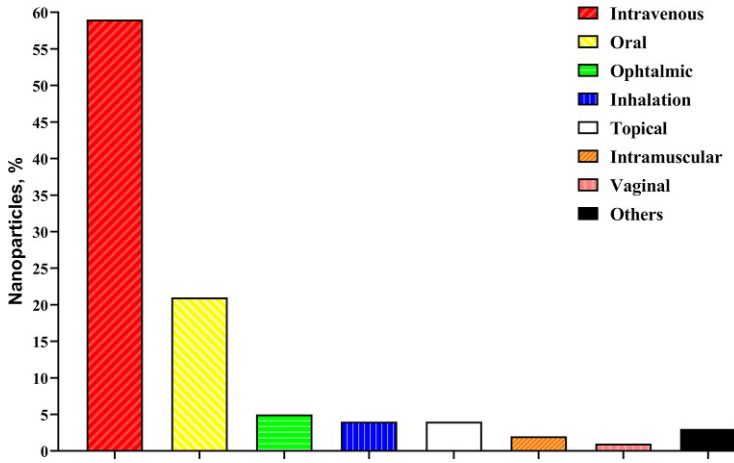

Fig. 1 Routes of administration of the various nanomedicines developed between 1973 and 2015. *Modified from S.R. D'Mello, C.N. Cruz, M.L. Chen, M. Kapoor, S.L. Lee, K.M. Tyner, The evolving landscape of drug products containing nanomaterials in the United States, Nat. Nanotechnol. 12(6) (2017) 523–529.*

and 75% of those individuals reported moderate, severe, or extreme pain [10–12]. Furthermore, more than half of the patients have reported inadequate pain relief [10]. The persistence of the pain for more than 3–6 months after the wound has healed is also experienced in 10%–50% of the patients who underwent routine surgery which constitutes a significant clinical problem [13, 14]. Postoperative pain is unique and is the result of a combination of nociceptive, inflammatory, and in some cases, neuropathic pain [13, 15] (Fig. 2).

The intensity of the pain is directly related to the magnitude of the nociceptive inputs perceived by the peripheral sensory neurons when an incision is made [15, 16]. The inflammatory pain refers to the increased peripheral and central sensitization due to the inflammation of the injured tissue [17]. The secreted inflammatory mediators decrease the nociceptor's sensitivity threshold and promote hypersensitivity known as allodynia and hyperalgesia [17]. The inflammatory pain is resolved when the inflammation of the tissue stops [13]. The surgically induced neuropathic pain arises from injury to nerves with paradoxical hypersensitivity as a consequence of the compression, transection, contusion, stretching, or inflammation of the nerve [18, 19]. In addition to the loss of sensation resulting from the nerve damage, some individuals experience spontaneous pain, dysesthesia, and hypersensitivity, including allodynia, hyperalgesia, and hyperpathia [13, 20]. Importantly, pain following surgery is not the consequence of a localized inflammatory event or single nerve damage but rather the combination of pathophysiological events.

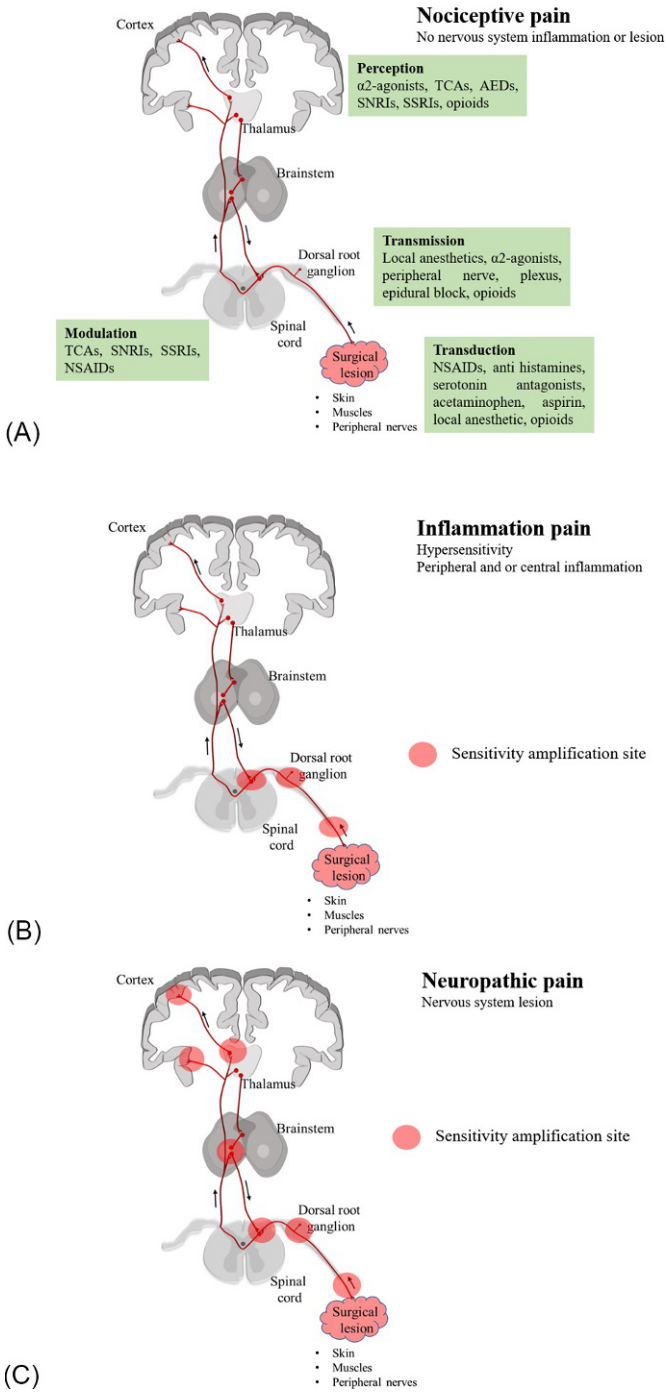

Fig. 2 Types of postoperative pains. (A) Nociceptive pain, (B) Inflammatory pain, and (C) Neuropathic pain. The four elements of pain processing (transduction, transmission, modulation, and perception) are shown in (A), as well as the drugs to modulate each element. Sensitivity amplification sites are shown in (B) and (C). *AED*, antiepileptic drug; *NSAID*: nonsteroidal antiinflammatory drug; *SNRI*, serotonin-norepinephrine reuptake inhibitor; *SSRI*, selective serotonin reuptake inhibitor; *TCA*, tricyclic antidepressant. *Adapted from M. Costigan, J. Scholz, C.J. Woolf, Neuropathic pain: a maladaptive response of the nervous system to damage, Annu. Rev. Neurosci. 32 (2009) 1–32.*

2.2 Treatment of postoperative pain

The mainstay for postoperative pain management involves the use of opioids to modulate the activity of nociceptors in the central nervous and peripheral tissues [21]. Morphine is the most commonly used opioid to treat acute postoperative pain, followed by fentanyl and hydromorphone, two more potent synthetic derivatives of morphine but with a shorter onset and half-lives [21]. The opioids can be administrated through different routes including intramuscular, oral, or transdermal but more frequently through intravenous injection to promote a rapid onset. Also, all opioids have numerous and severe side effects, for example, respiratory depression, postoperative vomiting and nausea, and pruritus [22]. Furthermore, the risk of persistent opioid use can lead to dependence and addiction [21, 23, 24]. The dose-dependent side-effects observed with opioids are often reduced by the combination with other analgesics to reduce opioids dosing. Nonsteroidal antiinflammatory drugs (NSAIDs) such as ibuprofen, naproxen, ketorolac, and cyclooxygenase-2 (COX-2) inhibitors have analgesic and antiinflammatory properties and are used either for short-term treatment of acute pain or as adjuncts of opioids in mild to severe levels of pain [22, 23]. Acetaminophen given either through oral, rectal, or parenteral route can reduce the intensity of the pain and is usually given in combination with opioids or NSAIDs [25, 26]. The treatment of neuropathic pain remains highly inefficient, opioids, and COX-2 inhibitors are efficient to decrease the inflammatory pain but not for the prevention of neuropathic pain [13]. Gabapentin, an anticonvulsant, and duloxetine, an antidepressant, are the two most commonly used agents for the treatment of postoperative neuropathic pain but have approximately 30% treatment efficacy [19]. The opioid use for the treatment of neuropathic pain is also tempered by the possible change induced in the brain [27] and aggravation of the neuropathic pain and hyperalgesia [28, 29].

2.3 Nanomedicine for the management of postoperative pain

2.3.1 Approved nanomedicine for the treatment of postoperative pain

The encapsulation of drugs into carriers has proven to increase their solubility, decrease the risk of systemic toxicity, improve their stability, and prolong their release when compared to the respective free formulation. Several analgesic nanoformulations were approved by the Food and Drug Administration (FDA) such as DepoDur and Exparel (Table 1).

The FDA approved DepoDur in 2004; the liposome formulation was designed for the extended release of morphine sulfate and used in postoperative pain management. The morphine encapsulated liposome provided pain relief for 48 h following epidural injection [30]. However, several adverse events similar to the administration of morphine have been reported, such as decreased oxygen saturation, vomiting, constipation, anemia, pyrexia, and pruritus [32].

Table 1 FDA-Approved Nanodrugs for analgesia.

Trade name	Manufacturer	Nanocarrier	Drug	Route of administration	Indication	Year of approval	References
DepoDur™	Pacira Pharmaceuticals	DepoFoam Liposome	Morphine sulfate	Epidural space	Postsurgical local analgesia	2004	[30]
Exparel®	Pacira Pharmaceuticals	DepoFoam Liposome	Bupivacaine	Infiltration	Postsurgical local analgesia	2011	[31]

DepoFoam bupivacaine (Exparel) was approved in 2011. Bupivacaine is a local anesthetic which encapsulation into a liposomal formulation improved its long-lasting activity as the analgesic effect is lasting up to 72 h. The bupivacaine DepoFoam formulation is administrated as a single dose infiltration into the surgical site [33, 34]. Several studies have demonstrated the potential of DepoFoam bupivacaine for the containment of pain following surgery [35]. The liposomal formulation was registered in 113 clinical trials, 41 studies were completed, and 44 are recruiting or active. DepoFoam bupivacaine was also assessed in several stage IV clinical trials for various surgical procedures (Table 2). The liposomal formulation was delivered mainly through infiltration. For most

Table 2 DepoFoam bupivacaine completed clinical trials stage IV.

Drug	Indication	Route	Clinical trial number	Completed
DepoFoam Bupivacaine	Arthroplasty, knee	Intracapsular injection	NCT02255500	2015
			NCT02166632	2017
		Periarticular injection	NCT02682498	2017
			NCT02274870	2017
		Infiltration	NCT02011464	2017
			NCT02284386	2016
			NCT02223364	2017
	Total abdominal hysterectomy	Infiltration	NCT02074709	2018
	Sternotomy, thoracotomy, or laparotomy	Infiltration	NCT02111746	2018
	Colon cancer surgery	Infiltration	NCT02052557	2014
	Open colectomy	Infiltration	NCT01507246	2013
	Ileostomy reversal	Infiltration	NCT01509807	2013
			NCT01509638	2013
	Open and laparoscopic abdominal hernia repair	Infiltration	NCT02128646	2017
	Unilateral abdominal hernia repair	Infiltration	NCT01801124	2013
	Cesarean surgery	Infiltration	NCT03176459	2019
	Bariatric surgery	Infiltration	NCT02142829	2014
	Shoulder surgery	Infiltration	NCT02472314	2019
	Anterior cruciate ligament reconstruction	Infiltration	NCT02606448	2016
	Tonsillectomy	Infiltration	NCT02444533	2017
		Infiltration	NCT02199574	2016
	Breast augmentation	Infiltration/ Instillation	NCT01582490	2014
	Hip fracture	Infiltration	NCT03289858	2019
	Total hip arthroplasty	Infiltration	NCT02242201	2018

of these studies, the results are still pending, but outcomes of NCT02052557 and NCT02959996 concluded that Exparel procured no advantage over the control formulations [36, 37], while others (NCT01507246, NCT01509807, NCT01509638) demonstrated significantly less opioid consumption, opioid-related adverse-effects, and better health economic outcomes [38].

Recent studies have also reported various drug delivery strategies of analgesic compounds through nonparenteral routes for the treatment of postoperative pain. Liposomes, polymeric nanoparticles, solid lipid nanoparticles, and other biocompatible, nonimmunogenic, and biodegradable polymers have been assessed in vitro and in vivo for the delivery of analgesics.

2.3.2 Nonparenteral delivery of analgesic encapsulated nanomedicine

The peripheral opioid receptors are important targets for the control of pain and inflammation following surgery [39]. Several opioid nanoformulations have shown to enhance drug delivery at these peripheral sites while minimizing the occurrence of side effects [40, 41]. However, only a few studies have reported the use of a nonparenteral route to achieve local postoperative analgesia. The mucosal route via nasal, oral, pulmonary has gained increasing interest over the past few years for the noninvasive delivery of analgesic encapsulated nanomedicines. The transmucosal delivery relies on the absorption of the nanomedicine into highly vascularized tissues to achieve a rapid plasma level and bypass the hepatic first-pass metabolism to increase the bioavailability.

The following are a few examples of nonparenteral delivery of analgesic encapsulated nanomedicines. The intravenous route of administration of fentanyl, an opioid, is the gold standard for postoperative pain relief. However, it is frequently associated with complications such as hypotension, bradycardia, and respiratory depression. The intranasal administration using a pressurized olfactory delivery (POD) device could be a promising noninvasive method for the systemic delivery of fentanyl. Hoekman et al. developed an integrin targeted liposomal formulation composed of phospholipids—1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and 1,2-dimyristoyl-sn-glycero-3-phosphoglycerol (DMPG) lipids to encapsulate fentanyl [42]. The integrin targeting ligand Arg-Gly-Asp (RGD) peptide sequence is a well-established peptide used to target endothelial and tumor cells for therapy and imaging [43]. Fentanyl-loaded RGD-liposomes were stable under aerosolization, enhanced the overall analgesic effects when compared to the free formulation as measured by the tail-flick test using Sprague–Dawley rats [42].

Other studies used a solid formulation of fentanyl citrate called oral transmucosal fentanyl citrate (OTFC), 50 to 100-fold more potent than morphine [44]. OFTC allows the delivery of fentanyl through the buccal mucosa to avoid first-pass metabolism and increasing bioavailability when compared to oral administration. OTFC has a rapid onset of action and short duration of effect [44]. OTFC was the first opioid analgesic developed

and approved for the control of breakthrough pain in cancer patients and has been assessed in several clinical settings independent of cancer-related pain [44]. Landy et al. evaluated the potency of OFTC vs morphine administrated by i.v. in postoperative analgesia. The study was conducted in 133 postoperative patients who received OTFC (200 or 800 μg), morphine (2 or 10 mg), or placebo (injection or transmucosal). OTFC (200 μg) demonstrated a similar effect as morphine, but the OTFC high dose produced better and more sustained analgesia [45].

Clark et al. reported the use of AeroLEF, a proprietary combination of free and liposome-encapsulated fentanyl administrated by pulmonary inhalation for the treatment of acute pain [46]. The efficacy of the formulation was assessed in phase II clinical trial (NCT00791804) using 19 postoperative patients following anterior cruciate ligament surgery [46]. The patients self-administrated AeroLEF via breath-actuated nebulizer to establish analgesia. The formulation was provided a rapid onset and prolonged analgesia compared to i.v. fentanyl, and well-tolerated with no serious adverse effects reported [46]. Javelin Pharmaceuticals has developed a chitosan-based nasal formulation of morphine (Rylomine) which is currently in phase III clinical trials in the United States and phase II clinical trials (the United Kingdom and European Union) [47]. Chitosan nanoparticles promote the mucoadhesion and drug absorption. The efficacy of Rylomine was compared to i.v. morphine and placebo in phase II clinical trial recruiting 187 post orthopedic patients (NCT00388011). The proportion of patients achieving ≥50% pain relief was 52.4%, 41.7%, and 80.4% for Rylomine 7.5, 15 mg, and i.v. morphine, respectively, compared to 21.7% for placebo, respectively. The median time to rescue medication was 124 and 140 min for Rylomine7.5 and 15 mg groups, respectively, and 130 min for i.v. morphine. The adverse effects of Rylomine were transient and mostly mild [48]. Rylomine was later evaluated in phase III clinical trial (NCT00390039), but no result is currently available.

Chronic postoperative neuropathic pain is experienced by 10%–40% of patients after common surgeries [19] usually as a consequence of inflammation or mechanical factors such as compression, stretching, transection, or contusion [18]. Neuropathic pain can be discerned from nociceptive pain by the absence of transduction, where the nociceptive stimulus is converted into an electrical impulse and is associated with the damage or disease of the somatosensory nervous system [49]. Neuropathic pain treatment efficacy is limited in the majority of patients due to the resistance to the opiate analgesic [50, 51]. While most of the nanomedicines developed to treat neuropathic pain are using the parenteral route for drug delivery [52]; few in vivo studies have reported formulations were developed for delivery through nonparenteral routes including oral, transmucosal, topical, and nasal.

Recently, Berrocoso et al. used poly(lactic-*co*-glycolic acid) (PLGA) coated with polyethylene glycol to encapsulate a potent synthetic cannabinoid receptor-1 (CB-1)/cannabinoid receptor-2 (CB-2) agonist 13 (CB13). PLGA is biocompatible, biodegradable

and improves the oral bioavailability of drugs with low solubility [53]. Cannabinoids agonists were shown to be efficient for the treatment of chronic pains, and CB13 was shown to reverse neuropathic mechanical hyperalgesia [54, 55]. The CB13-PLGA-PEG nanoparticles were administrated orally and maintained the analgesic effect for 11 days after a single dose while the free CB13 effect lasted only 9 h in a rat model of neuropathic pain using a chronic constriction injury [56].

Previously, Sukhbir et al. encapsulated nefopam into nanospheres structured by a mixture of Eudragit RL 100 and RS 100 with sorbitan monooleate as a surfactant [57]. Nefopam is a nonopioid, nonsteroidal centrally acting analgesic prescribed for the treatment of nociceptive and neuropathic pains [58]. The nefopam nanospheres were 328 nm in diameter and had a loading capacity of 21.4% [57]. The nefopam nanospheres were evaluated in a chronic constricted injury method in male Wistar rats. The nefopam nanospheres were administrated by oral gavage and achieved a significant difference in pain behavior from 2 h to 10 h postinjection as compared to nefopam rats [57].

Morphine is used to treat neuropathic pain. However, the chronic administration of morphine is associated with the development of analgesic tolerance, limiting its application in the clinic. The mechanism of tolerance is mediated through the activation of microglia and brain-derived neurotrophic factor (BDNF) release [59]. Curcumin was shown to abolish the upregulation of BDNF gene expression and reduce analgesic tolerance [60]. However, curcumin is characterized by poor bioavailability which has hindered its clinical development [61]. Shen et al. developed PLGA nanoparticles encapsulating curcumin and demonstrated that the oral administration of PLGA-curcumin nanoparticles restored morphine-antinociception as determined by mouse behavioral studies using tail-flick and hotplate assays [62].

Lalani et al. prepared PLGA nanoparticles loaded with tramadol hydrochloride and coated with two glycoproteins lactoferrin and transferrin to increase brain targeting [63]. Tramadol hydrochloride is a synthetic analgesic structurally related to morphine and codeine but less potent and acts on the central nervous system by exhibiting opioid and nonopioid properties [64]. Tramadol hydrochloride is characterized by its rapid metabolization and the encapsulation into PLGA-coated lactoferrin and transferrin nanoparticles, and delivery through the nasal route improved its pharmacokinetics and antinociceptive performance as measured the hot plate test using Wistar rats [63].

Dental surgeries require, in most cases, a preoperative local anesthetic and postoperative analgesia. Nidhi et al. have created a bilayer transmucosal patch incorporating lignocaine for a rapid release and anesthetic effect and diclofenac diethylamine solid lipid nanoparticles to ease the pain following dental surgery by the slow release of diclofenac diethylamine [65]. The solid lipid nanoparticles were prepared using Precirol ATO 5 and Geleol as lipids and Pluronic F 68 as the surfactant. The transmucosal patch was prepared with a hydroxypropyl cellulose-LF (HPC-LF) and with a backing layer of ethyl cellulose. The drugs were incorporated into the transmucosal patch and evaluated ex vivo using

porcine buccal mucosa and in vivo on the gingiva of New Zealand rabbits. The transmucosal patch displayed immediate release of lignocaine and sustained release of diclofenac diethylamine [65].

Despite the progress made into the nanoformulation of opioid and nonopioid for the management of postoperative pain [40], very few nanodelivery systems have progressed toward the nonparenteral delivery using oral, nasal, and pulmonary.

3. Wound healing

Wound healing has been described to occur in three continuous and overlapping phases [66]. The inflammatory phase involves blood coagulation and homeostasis, cellular infiltration to reduce infection, and cytokine secretion to promote cell proliferation. The proliferative phase is implicit of the epithelium covering the wound surface and growth of granulation tissue to fill the wound. The remodeling phase is the restoration of the functionality of the tissue [66]. Despite the use of appropriate antiseptic techniques, the complications related to postoperative wound healing up continue to occur with varying frequencies depending on the underlying disease. The infection of the surgical sites continues to be a significant health care burden as patients require hospital readmission, secondary surgeries, prolong hospitalization with a substantial increase in the cost of care, eventual disabilities, and morbidity [67]. Overall, postoperative infections occur in 1%–3.1% of patients and represent 2% of death associated nosocomial infection; for abdominal surgeries, nosocomial infections amount to 15%–25% of patients [68].

3.1 Wound dressing

The infections of surgical sites are the most common type of nosocomial infection observed in patients and are responsible for the delayed healing and potential dehiscence. The infection of the surgical site accounts for 14%–17% of all hospital-acquired infections and 38% of nosocomial infections in surgical patients [69]. Bacteria are the most common organisms responsible for the infections, and their proliferation and aggregation tend to form multilayers known as biofilms [70]. The management of the surgical wound is mainly dependent on the dressing materials and bandages. Wound dressings constitute a critical aspect of the prevention of infection for postoperative care and should maintain a moist environment to promote healing, remove excess exudate, and constitute a barrier against bacteria or other contaminants [71]. Nanomaterials have attracted much attention in recent years due to their wide range of applications in medicine and wound management. The size, versatility of shape, malleability of nanomaterials to make thin films, high surface to volume ratio, control pore size, lightweight, and mechanical strength are all characteristics applicable to wound healing [72].

The dressings can be classified in several ways as the type of nanomaterials used including metals, lipid-based, polymers, carbon-based or ceramic, or the physical form

of the dressing such as film, foam, or gel [73, 74]. Polymer nanoparticles made of biocompatible poly (lactide-*co*-glycolide) (PLGA), polycaprolactone (PCL), or polyethylene glycol (PEG) were used for the wound dressing and delivery of antibiotics [74, 75]. Choipang et al. develop polyvinyl alcohol (PVA) hydrogel containing ciprofloxacin hydrochloride (CIP)-loaded PLGA nanoparticles to treat infected pressure ulcers [76]. The created hydrogel displays antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* while showing no cytotoxicity [76].

Various metals nanoparticles have also been used for wound dressing to promote healing and to minimize infection such as silver. Silver has been used for centuries as antimicrobials agent. The silver cations are recognized antimicrobial agents at low concentration against a variety of pathogens but also eukaryotic cells which have limited the extent of his application in the clinic. Various nanoformulations of silver have been developed to improve the safe use against eukaryotic cells. Silver nanoparticles, sized from 1 to 100 nm, demonstrated a dose-dependence bactericidal effect against Gram-positive and Gram-negative bacteria [77, 78]. Also, silver nanoparticles showed antibacterial effects against antibiotic-resistant bacteria. The effect of silver nanoparticles on ampicillin-resistant *E. coli* and multidrug resistant *S. aureus* was independent of the acquisition of antibiotic resistance [79]. Furthermore, silver nanoparticles increased the sensitivity of multidrug-resistant bacteria isolated from patients and several pathological bacterial strains to various antibiotics [80, 81].

Silver nanoparticles (40 nm) were applied topically on laparotomy wounds following surgery performed on female New Zealand White rabbit [82]. The study demonstrated that the daily application of silver nanoparticles over 2 weeks decreased the bacterial load without systemic toxicity but promoted the formation of some local tissue edema [82]. Previous studies conducted on intact rabbit skin demonstrated that small size silver nanoparticles (10–20 nm) caused erythema and edema [83] while larger sizes (50 and 80 nm) tested on intact pig's skin did not cause any macroscopic irritation but only focal inflammation and edema [84]. Cohen et al. applied nanocrystalline silver to a propylene mesh and evaluated for its bactericidal effect in vitro [85]. More recently, ABL Medical developed SilvrSTAT; an FDA cleared as a 510(k) medical device; the silver nanoparticle-containing hydrogel was shown to be effective for the treatment of postoperative infections [86]. The combination of silver nanoparticles and chitosan was also tested in several preclinical studies in vitro and in vivo and demonstrated the potent bactericidal effect and increased healing rate [87–90]. Chitosan is a cationic polymer promoting the disruption of the bacterial cell membrane and its agglutination [91]. Carboxymethyl chitosan has been reported to be a broad spectrum antibiofilm polymer [92].

Silver nanoparticles were also demonstrated to decrease inflammation in a postoperative peritoneal adhesion model, thus affecting the expression of cytokines associated with the healing process (for review see Ref. [93]).

Table 3 List of clinical trials using silver nanoparticle preparation for postoperative wound healing.

Name	Indication	Clinical trial number	Phase	Status
ACTICOAT	Healing of wounds after curettage and electrodesiccation of skin lesions.	NCT01004055	2	Completed 2009
ACTICOAT	Poststernotomy wound healing.	NCT02198066	N/A	Completed 2014
ACTICOAT	Split-thickness skin graft.	NCT01769144	N/A	Recruiting
PolyMem Silver	Polymeric Membrane Dressing Plus Negative Pressure Wound Therapy	NCT02399722	N/A	Completed 2015
PolyMem Silver	Management of shave biopsy sites	NCT00727870	4	Unknown

Few clinical trials have assessed the effect of silver nanoparticles and nanocrystalline silver incorporated into wound dressing for the management of postoperative wound healing (Table 3).

Acticoat is composed of an absorbent polyester inner core sandwiched between outer layers of nanocrystalline coated polyethylene net; the concentration of silver is 0.84–1.34 mg/cm^2 [94]. PolyMem Silver is a polyurethane membrane matrix containing F68, glycerol superabsorbent starch, and silver nanoparticles, and the concentration of silver is 0.124 mg/cm^2 [94]. The antibacterial effect of these wound dressings was assessed, but the studies failed to provide a definite conclusion on the toxicity of silver nanoformulations following dermal exposure [95]. The silver released may accumulate in the skin, triggering argyria, as well as the kidney, brain, and heart [96]. All silver nanoparticle-incorporated wound dressing displayed significant cytotoxic effects against keratinocyte and fibroblast cultures and caused the significant delay of reepithelialization in the mouse excisional wound model after 7 days [97]. These observations led to recommendations to change the wound dressing after 1–7 days depending on the type of injury [98]. More clinical studies are required to determine the optimum exposure time to silver nanoparticles to achieve bactericidal effect while minimizing the cytotoxicity and rapid wound healing.

Other nanoparticles based on copper, graphene, titanium oxide, fibrin, polycations, and zinc oxide were also incorporated in wound dressings and assessed for their antimicrobial activities in vitro and in vivo [99]. Furthermore, recent studies have focused on the development of rapid wound healing approaches based on gene delivery using nanocarriers or the delivery of wound healing promoting factors such as nitric oxide using nanocarriers and were recently reviewed by Naderi et al. [96].

3.2 Corneal wound healing

Corneal wound healing is an important medical issue where nanomaterials have recently been applied. Postoperative corneal wounds arise from transplantations, incisions for cataract removal and intraocular lens implantation, and laser-assisted in situ keratomileusis (LASIK) [100]. Over 40,000 corneal transplantations are performed annually in the United States; the number of LASIK correction surgery is close to 20 million [101]. Complications with abnormal wound healing following LASIK surgeries are observed in 2% of patients [101]. Corneal wounds are usually repaired by nylon suture, but more than half of postcorneal transplant infections are related to complications with the wound closure [102]. Synthetic ocular adhesives were developed to minimize complications associated with the suture wound closure. Dendrimers are polymeric repetitive hyperbranched macromolecules and provide unique chemical and physical properties for corneal postoperative wound repair. Dendrimers are synthesized from amino acids, sugar, fatty acids, or metabolic intermediates and can be cross-linked to form an adhesive. Dendrimer-based adhesives were previously used to repair corneal perforations of 4.1 mm, to seal the flap originated in a LASIK operation, and to secure a transplant of cornea ex vivo [103–105]. Dendrimer formulations were further altered with polyethylene glycol (PEG), glycerol, polylactic acid (PLA), and succinic acid to improve the stability and biocompatibility of the adhesive [102].

3.3 Surface coating of orthopedic implant

The management of infection is an essential aspect of orthopedic surgery. Stainless steel, zirconium, cobalt chrome alloys, titanium, and their alloys have been used extensively due to their biocompatibility, low erosion, low cost, and mechanical strength [106]. The coating of the surface of these materials is to protect the implant from corrosion, reduce friction, promote its integration into the host tissue, and minimize the risk of infection [106].

3.3.1 Silver nanoparticles

Silver nanoparticles have been investigated for the coating of orthopedic implants to decrease postoperative infection rates. Silver bactericidal property is well established and acts by the inactivation of key enzymes by thiol group binding, the formation of reactive oxygen species, and DNA damage [107]. Albeit, the resistance of bacteria to silver nanoparticles develops slower than the one observed with antibiotics [108]. Silver nanoparticles immobilized on titanium prevented the formation of biofilm in vitro and in vivo [109]. The surface coating of titanium dental implants with catechol-functionalized chitosan and silver nanoparticles prevents the surface adhesion of bacteria while displaying negligible cytotoxicity [110]. The coating of titanium pedicle screw with polypropylene-based silver nanoparticles precludes the formation of bacterial biofilm

following implantation in the lumbar spine of New Zealand rabbits [111]. Pin tract infection is the most common complication associated with the use of fixators, the coating of the stainless steel pin with silver nanoparticles decreased biofilm formation in vitro [112]. Pishbin et al. developed a chitosan, bioglass particles, and silver nanoparticles orthopedic coating on stainless steel and assessed antibacterial effect against *S. aureus* and biocompatibility for the growth of osteoblast-like cells [113].

No implant coated with silver nanoparticles has been tested in the clinic. The concerns over the safety of the coating with silver nanoparticles remain a substantial limitation to its evaluation in a clinical trial. However, Wafa et al. conducted a case–control study to examine the benefice of the silver coating of endoprostheses on the prevention of infection [114]. The study reviewed the cases of 85 patients with silver-coated tumor implants compared to 85 cases of tumor implants lacking coating. The silver-coated implants decreased the rate of early periprosthetic infection and improved the success of the revision surgery [114]. Zajonz et al. conducted a retrospective analysis demonstrating that the silver coating of modular mega-endoprostheses on the hip and knee joint after healed periprosthetic joint infection reduced the rate of reinfection when compared to nonsilver coated modular mega-endoprostheses [115]. Additional studies will be necessary to ascertain the safety of silver nanoparticles for the coating of orthopedic implants and reduce the risk of infection.

3.3.2 Copper nanoparticles

The antimicrobial properties of copper have been known for centuries, and the recent development of copper nanoparticles has demonstrated potent antimicrobial activity against bacteria known to populate surgical wounds [116]. Albeit copper showed less cytotoxicity when compared to silver, few studies have been looking at its application of the surface of an orthopedic medical device [117]. Ren et al. developed a copper-coated stainless steel, which showed antimicrobial property by preventing biofilm formation [118]. Recently, copper nanoparticles were incorporated into titanium alloy layers and demonstrated antimicrobial activity in vitro [119].

3.3.3 Other inorganic nanoparticles

Iron oxide and zinc oxide nanoparticles have all demonstrated bactericidal activity [120, 121]. Both nanoparticles have been investigated in vitro to determine their antibacterial efficacy and their suitability as a coating agent for orthopedic surgery [120–123].

3.3.4 Chitosan

Chitosan coating of titanium alloys were also developed; several studies demonstrated antibacterial properties suitable for several medical devices [124–126]. Chitosan coating was investigated for the delivery of antibiotics such as tetracyclines and chlorhexidine digluconate; the chitosan-coated titanium pins were implanted for 7 days in the muscle

of Sprague–Dawley rats [127]. The study demonstrated the potential of chitosan for the delivery of antibiotics and its biocompatibility.

In addition to the few surface coating examples mentioned above, several preclinical studies are developing and testing nanomaterials and nanomedicine to improve the postoperative outcome of surgical implants. However, major hurdles still exist for the widespread of nanomedicines into clinical practice. The first consideration is the long-term exposure and impact of nanomedicine on human health that is not fully understood, incompletely study, and remains controversial in terms of negative consequences such as inflammation or positive outcome such as fostering faster healing. The second consideration is the cost associated with the development of such devices, their evaluation in clinical trials, and their marketing. Both concerns may limit further investments from biotechnological companies and prohibit new advancements from reaching the clinic [128].

4. Conclusion

The care of surgical wounds and management of associated pain has largely improved over the past decades due to the increasing understanding of the biological processes and the development of new therapeutic approaches. However, postoperative pain management is still far from being successful as many patients experience acute pain, which for many evolves to chronic pain. Furthermore, the analgesic treatment strategies, where limited adverse effects and risk of addiction are observed, are still missing. In recent years, a plethora of nanomaterials have been developed and demonstrated an improvement in the pharmacokinetics of various analgesic formulations while decreasing the incidence of side effects and minimizing potential addiction. However, only a few nanomedicines are approved by regulatory agencies and used in clinics worldwide. Most of the nanoformulations are still in the preclinical phase of development and augur future improvement in patient care. More recently, nanomaterials were developed and optimized for the delivery of analgesics through nonparenteral routes. Albeit, most of the studies are in the early stage of development, they demonstrated potent analgesic effects, lower incidence of side effects, and the perspectives of increased patient compliance and decreased healthcare cost. Also, nanomaterials were optimized to promote wound healing and to reduce the risk of infection. The applications of these nanomedicine formulations are mainly topical and demonstrated antimicrobial activity while preserving the integrity of the cells and promoting rapid healing. Future clinical studies will be required to assess the long-term toxicity effect of these topical nanomedicines and to tease apart the toxicity emanating from the nanomaterial and toxicity arising from the drug in humans. Overall, it will be of paramount importance to understand pathophysiological variabilities among patients to determine the most appropriate treatment.

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CHAPTER 19

From barriers to bridges; glycans in nonparenteral nanomedicines

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1. Introduction

Biological barriers perform the primary function of mediating passage of molecules in and out of organs. Their complex structure and physiology allow them to provide selective passage of some substances, while also preventing the penetration of pathogens and undesired substances. In this way, biological barriers can manage the availability of a variety of molecules with efficiency and fidelity that has yet to be matched by any man-made filter or barrier. Without such a barrier, our lungs, for example, our bodies would be vulnerable to a range of dangerous airborne molecules that could enter the bloodstream and affect all of our organs. Most delicate of all, the integrity of the brain would be compromised in the face of an infection or other challenges.

The success of most biological barriers lies in the composition of their membrane; consisting of tightly joined epithelia backed up by an array of other cell types involved in processes such as clearance and immunity. While some small molecules can diffuse or passively partition across these barriers, the majority of molecules and other undesirables, are removed by active biological processes. Larger molecules that do pass through biological barriers must traffic across using active energy-dependent mechanisms that expose these molecules to many intracellular checkpoints [1].

Nanoparticle-based drug-delivery systems have been revealed as suitable vehicles for overcoming pharmacokinetic limitations that conventional drug formulations have faced [2]. Nanoparticles, such as liposomes, have proven advantageous at solubilizing therapeutic cargos, extravasating the drug to disease sites, and substantially prolonging the circulation lifetime of drugs [3]. These properties have proven largely beneficial in enhancing the safety and tolerability of nanoparticle-formulated drugs; best shown by the reduced cardiotoxicity observed in patients administered liposomal doxorubicin, relative to conventional formulation approaches [4]. Improvements in patient morbidity following the use of liposomal doxorubicin led the US Food and Drug Administration to approve nanoparticles for the

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treatment of Kaposi's sarcoma in 1995 (Doxil) [5, 6]. In addition, the use of nanoparticle albumin-bound paclitaxel (Abraxane) was approved 10 years later, which resulted in reduced side effects compared with conventional paclitaxel formulations by eliminating the excipient Cremophor EL [7, 8]. Although improvements in patient safety and morbidity led to clinical approval of nanoparticle platforms, the efficacy of drug delivery in patients still remains modest, with only marginal improvements being observed relative to conventional formulations [6].

Although nanoparticles improve the circulation half-life of conventional drugs and increasing the chance to accumulate at the injury site, they face a complex series of biological barriers that may severely limit their therapeutic outcomes. Depending on the route of administration, target tissue, and state of disease progression (i.e., early- versus late-stage cancers), the obstacles in controlled drug delivery can vary. These include nonspecific distribution, sequestration by the immune system, hemorheological/blood vessel flow limitations, cellular internalization, escape from endosomal and lysosomal compartments, and exocytosis via drug efflux pumps [1]. Moreover, nonparenteral drug-delivery systems (including sublingual, aerosolized buccal, and intraocular) are exposed to additional barriers that hinder their effectiveness, such as mucosal barrier (Fig. 1) [1, 6, 9].

In this chapter, we review some of the biological barriers that drug-delivery systems face, focusing on the effects of the mucosal barrier on nonparenteral nanomedicine and the role glycans play in these phenomena. We also make reference to nanoparticles and how they are used to overcome some of the obstacles formed by biological barriers.

2. Biological barriers challenging drug-delivery systems

Biological barriers can take a range of forms throughout the body; they may be physical structures such as the skin, or more subtle factors such as the fluid dynamics in blood vessels. In some instances, these barriers consist of living cells (i.e., blood-brain barrier), whereas in others they consist of aqueous solutions (i.e., the acidic environment of the stomach) [1, 6]. Depending on the nature of a biological barrier, the mechanism required to overcome this obstacle and successfully deliver a therapeutic compound can vary considerably (as summarized in Fig. 1). For example, manipulating the physicochemical properties of a drug carrier would help overcome cell-free biological barriers, whereas targeting receptor-mediated mechanisms would help to overcome barriers that are composed of cells [1].

Nanoparticles have been developed to address a wide range of problems in pharmacokinetics that result from biological barriers. From a pharmaceutical perspective, nanoparticles are typically defined as particles less than 1 μm that contain an active pharmaceutical ingredient [10]. Nanoparticles that are traditionally used for drug delivery include liposomes, polymeric nanoparticles, micelles, dendrimers, and inorganic nanoparticles made of iron oxide, gold, and quantum dots [2, 11]. Nanoparticles have been

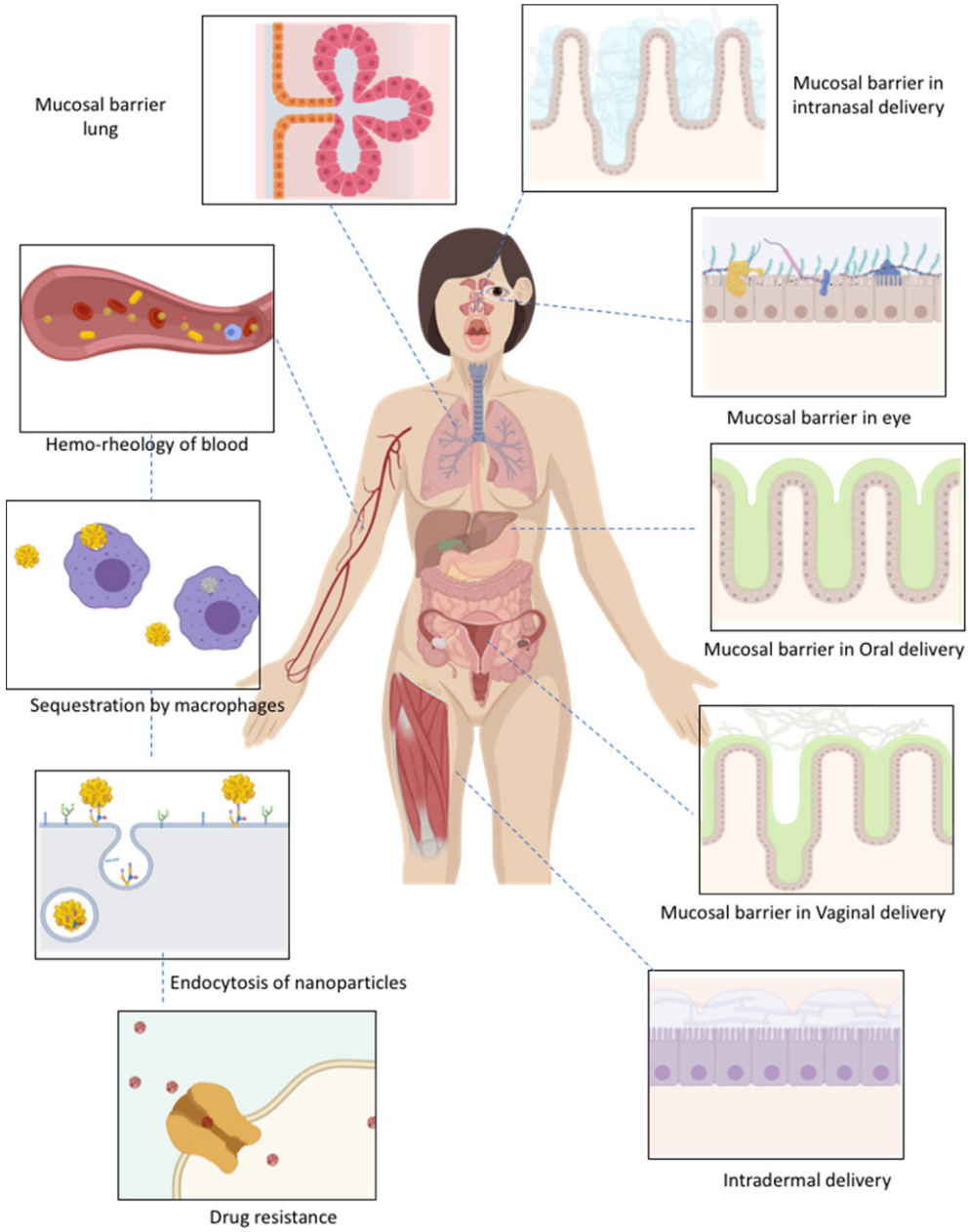

Fig. 1 Schematic illustration of nonparenteral administration routes and the biological barriers that challenge nonparenteral drug-delivery systems. In all of the biological barriers, glycans are key component of the obstacle or play important role in the physiology of the barriers.

found to significantly improve the solubility and circulation half-life of loaded drugs in the body. Although these modifications have highlighted the impressive versatility and preclinical potential of nanomedicine, very few nanoparticles have been successfully translated into clinical use [12, 13]. This has led many experts to question and challenge the ability of nanoparticle-based drug-delivery systems to achieve positive clinical outcomes. It must be noted that the physicochemical properties of nanoparticles that result in improved water solubility, circulation half-life, and targeted/controlled delivery are also known to create new challenges as well, such as penetration through cell membrane. Therefore, the minimal therapeutic impact observed directly correlates to the inability of nanoparticles to overcome biological barriers.

Due to several advantages, such as simplicity and local delivery, nonparenteral drug administrations are the most readily accepted drug delivery systems [9, 14]. However, poor stability in the gastric environment such as low solubility and overcoming the mucus barrier can prevent drug penetration and subsequent absorption. For most nonparenteral drug administration routes (including oral, nasal, ocular, vaginal, and anal) the epithelial glycocalyx and mucosal surface form the most important biological barriers limiting the bioavailability of drugs. These mucosal layers can create hurdle for drug bioavailability through adhesion mechanisms and steric hindrances [9, 15].

3. Mucous and the mucosal membrane as a barrier against nonparenteral drug delivery

The mucous membrane contains approximately; 95% (w/*w*) water, 0.2%–5.0% (w/*v*) mucins, 0.5% (w/*v*) globular proteins, 0.5%–1.0% (w/*w*) salts, and finally 1%–2% (w/*w*) lipids, DNA, cells, and microorganism [16–18]. The membrane is composed of one or more layers of epithelial cells that line various cavities throughout the body and covers the surface of internal organs. The human body contains over 400 m^2 of mucosal surfaces, making it the largest contact area with the external environment [19]. The mucosal membrane consists of a loosely adhered outer layer, and a firmly attached inner layer. The thicker, outer layer has been reported to be responsible for creating a barrier against the submucosal transport and release of drug molecules [20]. The diffusion of proteins and peptides confirmed this outer layer as the main factor that restricts the bioavailability of drugs intended for oral delivery [15]. Contrastingly, the inner mucosal layer has been found to enhance the absorption of drugs, indicating a dual role of this membrane in nonparenteral drug delivery systems [15, 21].

Mucous lining the mucosal membrane assists in mucociliary clearance, hydration, nutrients, and gas exchange, lubricating the epithelia, as well as protecting the internal environment from pathogens and foreign substances [15, 22]. In addition, disruption to the normal functioning of mucous plays an important role in the pathology of a wide range of diseases including cystic fibrosis, bronchitis, asthma [23], and cancer [24].

Mucous is also known to yield specific properties (i.e., rheological factors, charge, ionic strength, acidity, and pore size) that can suppress the delivery of drugs and other bioactive molecules [15, 25].

Approximately 10 L of mucus is secreted within the GI tract daily, making this a major determinant of nutrient absorption [9, 26]. The final thickness of the mucosal layer is a result of an equilibrium between secretion, degradation, and clearance [27]. The permeability of the GI tract is modulated by critical filtering properties of the mucus, with a variable disposition in different sections of GI such as the stomach, colon, and small intestine [9, 28]. In the stomach and colon, there is a thick mucus layer that protects the epithelium from acidic conditions and pathogens. In the colon, the inner mucus layer is continuously renewed by global cells with a turnover time of 1–2 h [28]. The thinner mucus layer of the small intestine contributes to the adsorption of more than 90% nutrients, whereas the remaining 10% is absorbed in the stomach and large intestine [16, 18].

4. The structure of the mucosal barrier

Mucus is a viscous colloid consisting of immunoglobulins, inorganic salts, antimicrobial enzymes, glycoproteins, and mucins. The viscoelastic properties of mucous are dependent on several forms of interactions; such as hydrophobic and hydrogen bond, covalent and noncovalent, and electrostatic interactions. Collectively, these properties contribute to the formation of a complex and dense network of mucosal fibers, which decreases the diffusion and penetration of particles and molecules [15].

The concentration and hydration state of mucin is also known to influence the viscoelasticity of mucus [29]. Mucins are negatively charged glycoproteins produced by submucosal glands and epithelial cells, with molecular weights in the range of 10–40 MDa [30]. Mucin fibers contain O-linked glycoproteins with a repeated domain of proline, threonine, and serine (PTS), giving them a high affinity toward particles with a positive charge [31]. Mucins are also known to be highly glycosylated (more than 70% of the protein's density), involving *N*-acetylgalactosamine (GalNac), galactose (Gal), fucose, sialic acid, *N*-acetylglucosamine (GlcNac), and low amounts of sulfate and mannose [30]. Many of these glycosylated forms of mucin are secreted by goblet cells and can form gels known as gel-forming (MUC2, MUC5AC, MUC5B, MUC6, and MUC19), or nongel forming mucins (MUC7, MUC8, and MUC9) [30].

5. Mucoadhesion and nanoparticle interactions

The interaction between the mucosal membrane and any given material (biological or synthetic) is defined as mucoadhesion; a process that has various applications for drug-delivery systems. Due to the rapid production and secretion of mucus, it is difficult for very small nonmucoadhesive nanoparticles to diffuse through this membrane

(Fig. 2). For example, rapid mucus production in the stomach and eyes results in topically applied nanoparticles being washing away. Moreover, this barrier becomes more difficult to overcome in areas like the small intestine, where epithelia yields more adhesive properties to maintain a dynamic flow of mucus [32].

Mucoadhesion between the mucosal membrane and drug delivery systems requires a number of interactions between mucins and mucoadhesive polymers. In brief, the process can be divided into two phases. Initially, the region of the mucosal membrane that comes in contact with the drug construct will undergo a swelling and expansion phase, forming an irregular network between the two mediums. Secondly, chemical interactions such as ionic, covalent, hydrogen, and electrostatic bonding occur between the two substrates (i.e., mucosal proteins and drug construct) [33]. The interactions of mucins are largely influenced by the type of polymer with parameters such as charge, hydration, chain flexibility, average molecular weight, and hydrogen bonding capacity [34]. Also, parameters affecting the buccal environment (i.e., ionic strength and pH) can also influence adhesion and residence time of the mucoadhesive doses [9, 33].

Drug-delivery systems targeting mucoadhesive can be also divided into specific and nonspecific delivery systems [33, 35, 36]. The concept of nonspecific mucoadhesion is a well-studied phenomenon dating back to 1962 when intestinal mucus from animals was used as a coating system to prolong the exposure time of orally administered particles [33, 37]. These findings provided the foundation for further research to explore mucoadhesion and generate novel strategies to improve the nonspecific adherence to mucins. Such strategies include the use of electrostatic interactions, hydrophobic forces, polymer chain interpenetrations, van der Waals interactions, and hydrogen bonding [33].

The incorporation of cationic materials within mucins is one approach for promoting an electrostatic interaction with anionic glycosylates, thereby enhancing mucoadhesion (Fig. 2). Chitosan is an important example of cationic polymer that has commonly been used in the formulation of mucoadhesive particles [38, 39]. Charged chitosan microparticles are prepared through spray drying, with the mucin adsorption ability of the microparticles being tested at various temperatures, pH, and ionic conditions. The extent of mucin adsorption was found to be proportional to the microparticles charge properties, specifically the chitosan positive zeta potential charge and the negative potential of glycoproteins [40]. In another example, cationic chitosan was used to coat the surface of negatively charged PLGA, enabling the chitosan-coated particle to exhibit enhanced mucoadhesion and improved in vitro cellular uptake compared to uncoated particles [41]. In another study, thiol groups were incorporated into the side chains of chitosan, forming in situ cross-linking through covalent disulfide bonds with the subdomain of mucus glycoproteins. This improved the mucoadhesion properties of the particles by a 140-fold relative to unmodified forms of chitosan [38, 42].

Common glycans (such as chitosan, alginate, pectin, and derivatives of cellulose) have been widely evaluated in their use as mucoadhesive polymers. To date, they have been

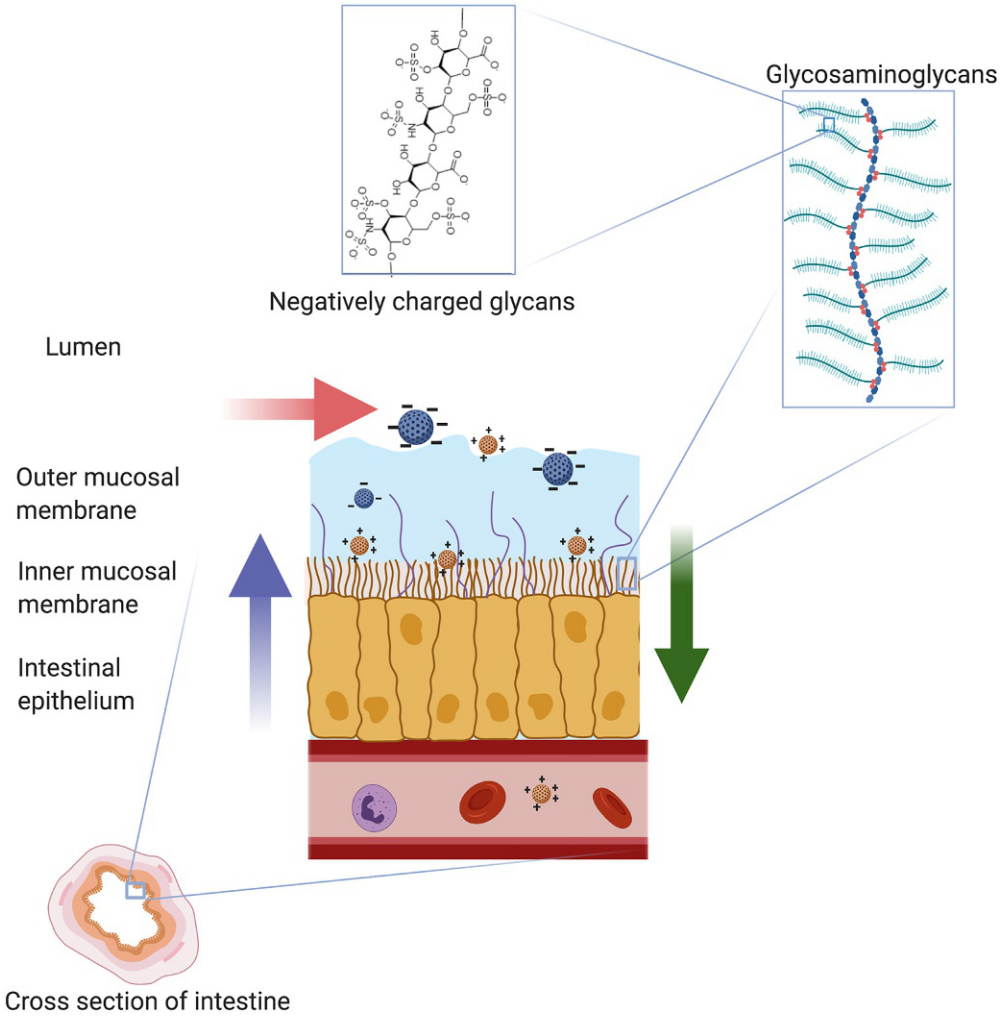

Fig. 2 Schematic illustration of mucosal barriers in oral drug delivery. The mucosal membrane consists of a tightly adherent membrane (inner mucosal membrane) and an outer (loosely adherent) membrane. For oral absorption of nanoparticles, the particles are required to interact and diffuse through the secreting mucus. *Rightward arrow* shows the movement of food and orally taken particles through GI, the *upward arrow* represents the constant secretion of mucus that is against the penetration of particles through the mucosal membrane (*downward arrow*). Particles, depending on their adhesive properties, have different bioavailability. Larger particles (in micron range) may not be able to diffuse through the mucus layer due to steric hindrance. The diffusion of smaller particles (200–500 nm) through the mucus layer depending on adhesive properties. For example, particles with positive surface charges are mucoadhesive and diffuse through luminal/external layers of mucus slower than others. The presence of highly sulfated and carboxylated glycans on mucus plays key role in the mucoadhesion phenomenon.

investigated not only in their pure form, but also in modified forms with other mucoadhesive polymers including PEG [43–46], polymethacrylates [47, 48], and poly(sebacic acid) [49, 50]. These polymers may improve mucoadhesion through hydrogen bonding, polymer-mucin entanglements, or a mixture of these mechanisms [36, 51].

The process of polymer-mucin entanglement was first introduced by the Peppas et al. producing pH-responsive hydrogels with a highly cross-linked structure for oral delivery of proteins [45]. Branching PEG polymers from the hydrogel were found to swell, interpenetrate, and entangle with the mucin fibers. The incorporation of linear PEG molecules into the hydrogels, therefore, significantly enhanced the contact time of PEG diffusion within the mucus gel [52].

An alternative approach for enhancing mucoadhesion is to attach specific binding ligands to the nanoparticle, thereby enhancing its ability to bind to mucin glycoproteins or epithelial cells. Lectins are abundant, naturally occurring proteins that can form reversible binds with specific sugars [53]. The expression of nanoparticles in the circulatory system after oral administration has been shown to increase 50-fold when conjugated with tomato lectin (TL) [54]. Nevertheless, many factors limit the use of mucoadhesive nanoparticles for oral delivery, such as possible adhesion to an unintended surface. Furthermore, nanoparticles can be trapped in loosely adherent mucus, which may result in rapid rather than slow controlled clearance.

A number of in vitro and in vivo studies have conjugated lectin wheat germ agglutinin (WGA) onto nanoparticles and reported significantly higher transepithelial uptake and improved drug efficacy in intestinal enterocytes [55–58]. For example, WGA-modified liposomes (approximately 200 nm in diameter) have been used as a peptide drug model to demonstrate the oral delivery of calcitonin in rats. The WGA-modified liposome showed higher adsorption and longer calcitonin circulation time in vivo, *as well as* a 20-fold higher plasma calcitonin concentration when compared to nonmodified control liposomes [57]. Nevertheless, the premature binding of these lectin-conjugated particles to the carbohydrate residues of mucins was found to negatively impact their distribution across the underlying epithelium, impacting their application [33, 59–65]. Collectively, a number of lectin-conjugated particles have been shown to improve interactions and specificity to porcine mucins [60, 65].

In order to increase the time of nanocarriers at mucosal surfaces, researchers have attempted to manipulate nanoparticle-mucin interactions in the hope of maximizing interactions with mucins. This strategy could result in extended transit and retention times of the nanoparticles resulting in higher drug bioavailability. This prolonged delivery would match the mucus renewal rates in the GI tract that is typically in the order of hours [34, 66].

Originating from viral research into mucosal penetration [67], another approach for improving the bioavailability of nonparenterally administered nanoparticles is via covering the particle with a muco-inert coating. Cone and coworkers discovered two capsid viruses (Norwalk virus and human papillomavirus) that readily diffuse through mid-cycle

endocervical mucus, these properties being attributed to their small size and inert surface [67]. The absence of glycans at the surface of the viruses may be a possible explanation to these muco-inert properties. A lack of glycans results in less carboxyl and hydroxyl groups at the surface, thereby reducing the degree of hydrogen bonding that occurs with mucins [33]. It has been shown that particles coated with hydrophilic, uncharged polymers could minimize adhesive interactions with mucins in a similar manner. As previously mentioned, PEG moieties at the surface of nanoparticles may be a good example of this [68].

6. Penetration to the target cells

Although some small synthetic nanomaterials (i.e., molecules, metal nanoclusters, small dendrimers, and carbon nanotubes) are able to pass passively through cell membranes, the majority of synthetic materials are larger than a few nanometers, and therefore require assistance from active uptake mechanisms (i.e., endocytosis or pinocytosis) [69]. For this reason, the endothelial cell membrane acts as an important barrier for most nanotherapies. To overcome this barrier, scientists have investigated the human body's intrinsic mechanisms that allow molecules to cross or penetrate the cell membrane [69].

Apart from size, another major factor that influences the ability of molecules to penetrate cell membranes is surface charge of the particles [6, 69, 70]. Various endothelial [70] and cancer cell types [71, 72] have been used to illustrate how cationic molecules (such as quantum dots or dendrimers with a positive charge) are able to create transient pores in cell membranes. This phenomenon occurs due to the electrostatic interactions between negative glycans and phospholipids on cell surface and positive moieties of the particles. While this does assist the movement of nanoparticles into cells, it is associated with increased cellular toxicity, questioning its appeal for nanotherapeutic applications [73].

Receptor-mediated endocytosis facilitates the ability of monotherapies to penetrate cell membranes [58, 74]. Carbohydrate-containing macromolecules, such as glycoproteins, have been found to cross cell membranes via specialized membrane-bound lectin receptors. The first membrane-bound lectin receptor discovered was the hepatic asialoglycoprotein receptor (ASGP-R) that recognizes glycoconjugates containing terminal galactose or *N*-acetylgalactosamine residues [75]. Lectin receptors have since been used as a classical receptor system for studying receptor-mediated endocytosis [76]. A wide variety of soluble and membrane-bound lectins have since been discovered and characterized in animals [76–78]. Membrane-bound lectins often contain endocytosis motifs within their cytoplasmic domains, such as the tyrosine-containing sequences found in mannose 6-phosphate [79], mannose, and ASGP-R [80]. These endocytic motifs have been found to recruit adaptor proteins, causing the formation of clathrin-coated pits and subsequent internalization of the formed vesicles [76]. The hyaluronan/chondroitin sulfate receptor for endocytosis (HARE) is another membrane-bound lectin that has recently been identified and discovered to be responsible for the turnover of glycosaminoglycans, an important component of the extracellular matrix [81].

7. Hemorheology and blood flow dynamics

Margination dynamics, or the drift of nanoparticles to the wall of blood vessels [82], is a very important nanoparticle design consideration. Enhancing the interaction of nanoparticles with endothelial walls is known to facilitate particle-cell binding and receptor-ligand interactions. Small spherical particles (i.e., liposomes) are found in a particular region of the vessel known as the cell-free layer, which is a direct result of nanoparticle-bound red blood cells preferentially accumulating within the core of a vessel [6, 82]. It is of note that this hemorheological characteristic plays a minor role in the site-specific delivery unless specific external forces (i.e., magnetic guidance) are applied [83].

The endothelial glycocalyx sit between the blood and the endothelial cell wall regulating cell adherence to a permeable barrier, interacting with a range of plasma proteins and lipids to regulate cell adherence and form a permeable vascular barrier [84]. The glycocalyx is worn down by flowing plasma and maintained by endothelial secretions, making it vary in thickness ranging from 0.1 to 1 μm [84–86].

The main constituents of the glycocalyx are glycoproteins and proteoglycans [84]. Proteoglycans have a protein core, and attached to this are many negatively charged glycosaminoglycan (GAG) side chains. The GAGs give the glycocalyx a negative charge, allowing it to repel molecules such as red and white blood cells that are also negatively charged while attracting positive molecules [87]. Proteoglycans have varying sizes, numbers, and lengths of GAG chains allowing them to be extremely diverse. Proteoglycans can be bound to cell membranes (i.e., syndecan) or present in the extracellular space (i.e., perlecans) allowing them to play a role in many diverse cell functions [88]. There are five different types of GAGs: heparan sulfate, chondroitin, dermatan, and keratin sulfates or hyaluronic acid. Hyaluronic acid differs from other GAGs as it has no core protein [89]. Of note, hyaluronic acid differs from the other GAGs as it is not usually bound to a core protein and tends to form viscous solutions with water [89].

The size and shape of a nanoparticle impact its fluid dynamics in the blood. Liposomes and polymer nanoparticles are usually spherical and are more conventionally designed for intravenous delivery. Nanoparticles of this design will undergo margination before adhering to the epithelial cell walls and becoming internalized. The surface properties of nanoparticles play a crucial role in the protein absorption. Highly positive nanoparticles are cleared fast from the circulation, being attracted to the endothelial glycocalyx for adherence and internalization [90]. The opposite is observed with neutral and negatively charged nanoparticles showing a significantly prolonged half-life in the circulation [91]. An investigation into the manipulation of the charge of nanoparticles in tumors, shows an increase in the accumulation of nanoparticles with zwitterionic surfaces [92]. Using these nanoparticles to deliver therapeutic interventions may be a useful tool in cancer research. The size of the nanoparticle carrier may also manipulate its functional ability, with larger particles accumulating in the spleen and liver [93].

8. Sequestration and the mononuclear phagocyte system (MPS)

The inability of nanotherapies to provide therapeutic levels of active drug to the target site remains a major limitation of these treatments. Nonspecific uptake of nanoparticles by nonspecific, healthy organs has been identified as a major contributor to this effect. The mononuclear phagocyte system (MPS) has been found as another mechanism that absorbs [6, 94]. The MPS consists of phagocytic cells (mostly resident macrophages) from the spleen, lymph nodes, and liver.

The MPS absorbs nanoparticles through opsonization and sequestration. First, various proteins from the plasma (i.e., serum albumin, apolipoproteins, complement components, and immunoglobulins) interact with the surface of circulating nanoparticles [95]. This interaction results in the absorption of these proteins and formation of a protein layer that surrounds the nanoparticle, known as the protein corona. The formation of the protein corona is regulated by a number of factors, including surface chemistry, nanoparticle size, hydrophobicity, and surface charge [6, 95, 96]. Once this protein layer has been formed opsonization occurs, a process in which immune cells target foreign substances for degradation. Specifically, the absorbed proteins expressed on the protein corona of nanoparticles have been found to bind to specific receptors on the surface of phagocytes, causing them to be internalized, transported to phagosomes and fused with lysosomes [97]. In addition, the absorbed proteins in the protein corona are also known to mask underlying ligands, causing a marked reduction in the specificity of nanoparticle binding sites. In summary, nanoparticles have proven to be a tunable, drug-delivery modality, warranting future work to properly understand how best to overcome the obstacles that the MPs create in the hope of maximizing their therapeutic application.

In the process of differentiating between self and foreign cells, oligosaccharides are covalently bound to surface proteins on cell membranes. Healthy mammalian cells can be recognized by immune cells investigating their surface due to their branched, O-linked core glycans containing high levels of *N*-acetyl-d-glucosamine (GlcNAc, such as sialic acid) [98]. Many pathogens show a limited expression of sialic acid allowing our immune system to recognize these glycan variations and respond to them accordingly [99]. In addition, the presence of some small glycan moieties (particularly, mannose, fucose, and sialic acids on the surface of foreign entities) play a key role in the innate recognition of pathogens [100]. As well as polysaccharide constitution, density, and spacing of glycans are important elements that dictate their interaction with immune cells and foreign entities. Larger glycans tend to disrupt this process, with heavily glycosylated glycoproteins forming a common escape strategy for enveloped viruses [101, 102]. However, protective glycosylation can be detrimental, becoming too dense and forming unique clustered epitopes that can be recognized by specific antibodies [103, 104].

These properties of glycans in immune recognition draw attention to the potential of glycan ligands for targeting glycan-binding proteins [105, 106] or for avoiding

recognition by immune cells. For example, a sialic acid-binding immunoglobulin-like lectin (CD22) has been widely used for the in vivo targeting of B lymphoma cells [107, 108]. Recently Tokatlian et al. developed nanoparticles from two heavily glycosylated human immunodeficiency virus (HIV) antigens; specifically, from a Gp 120-derived mini protein and a large stabilized envelope trimer. These particles were engineered to deliver nanoparticles to follicular dendritic cells (FDC) [106]. Gp 120 protein is known to mediate the attachment of HIV virus to glycosylated cell-surface receptor molecules (i.e., CD4 antigens). Supporting this, nanoparticles expressing Gp 120 proteins were found to be rapidly shuttled to the FDC network and concentrated in germinal centers [106]. Similarly, the decoration of nanoparticles with sialic acid enhances their ability to hide from immune systems, thereby enhancing their chances of reaching target cells and promoting their uptake [109–111].

9. Drug resistance and efflux pumps

The body's natural resistance to drugs represents a major hurdle for disease treatment. Multidrug resistance (MDR) refers to an intrinsic or acquired (through prolonged exposure) antimicrobial resistance shown by an organism toward multiple drugs, typically resulting in the efflux of drugs and a reduction in their therapeutic dose and impact. Classic MDR is conveyed via the action of ATP-dependent transporters which break down ATP to expel drugs out of the cell [6, 112]. However, dysfunctional apoptotic signaling pathways and activation of detoxifying systems have been found to underlie multifactorial forms of MDR [6].

Resistance to chemotherapeutics by malignant cells has proven to occur regardless of drug structure or mechanism of action. A clear example of this is shown through the use of anthracyclines, taxanes, and vinca alkaloids [113]. As a result, most chemotherapies require high treatment doses that can lead to toxic circulating levels of drugs and increased patient morbidity. Moreover, these therapies also result in increased cellular toxicity due to elevated exposure of the expelled drug to healthy cells.

Glycosyltransferases (GT) are a large family of enzymes that glycosylate proteins and lipids to produce glycoconjugates. Glycoconjugates are known to mediate several biological processes including detoxification, molecular transport, cell signaling, cell recognition, and cell adhesion [114]. Clinical and preclinical findings have shown the association between abnormal expression of GTs, aberrant glycosylation patterns, MDR, tumor proliferation, invasion, and metastasis [114–117]. Some examples of abnormalities in glycosylation include increased overall O-GlcNAcylation, sialylation of intracellular proteins, and UDP-glucose ceramide glycosylation [116]. These abnormalities were found to activate the protein kinase B (PKB), and ERK1/2 signaling pathway, leading to increased gene expression of MDR protein1 [115, 118].

10. Drug delivery to the central nervous system (CNS)

Compared to other systems in the body, the central nervous system (CNS) has additional challenges for drug delivery. In addition to the biological barriers mentioned previously, nanoparticles must pass through the blood-brain barrier (BBB), a sophisticated biological barrier separating the CNS from the peripheral environment.

The BBB is essential to maintain the unique neuroparenchymal environment of the brain, however, it limits the penetration of large molecules like nanoparticles, antibodies, recombinant proteins, or gene therapeutics [119]. The BBB is composed of four main cell types: endothelial cells, pericytes, astrocytes, and microglial cells. The tight junctions between the endothelial cells limit the permeability of the barrier, leaving very few drugs (i.e., some hydrophilic drugs, small proteins, and charged molecules) to passively cross the BBB [120]. The passive diffusion of the molecules, proteins, and drugs across the BBB depends on their physicochemical properties such as their structure, molecular size, charge, hydrogen bonding potential, and lipophilicity. It is worth noting that even though some compounds have successfully penetrated the BBB, their efficacy remains challenging due to active efflux mechanisms such as P-glycoproteins (P-gp) that continuously pump the compounds back into the blood [119, 121].

Several pathological conditions including cancer [122], stroke [123, 124], and trauma [125] are known to increase the permeability of the BBB transiently. This provides a unique opportunity for the delivery of particles to the target site within the CNS. In other circumstances, invasive brain-drug delivery systems have been used to overcome the BBB. These systems aim to create a transient increase in the brain-blood flow and/or BBB leakage via intracarotid arterial infusion of hyperosmotic solutions [126], noxious agents (including vasoactive compounds, [127]) or local ultrasonic irradiation of the brain [128, 129]. However, such approaches are also known to increase leakage of plasma proteins like albumin and bilirubin into the brain, leading to edema and toxicities [130].

Despite the limitations of drug delivery to the brain, biodegradable polymeric nanoparticles, such as colloidal carriers, have shown promise in several preclinical studies [92]. Successful drug delivery strategies for the treatment of neurological disease rely on increased bioavailability through intranasal and/or oral administration, and longer circulation half-life of the drugs. The hydrophobicity of drugs such as haloperidol, chlorpromazine, perphenazine, promazine, and quetiapine [131–133] limits BBB passage and often results in rapid elimination prior to absorption [92] (Fig. 3). Drug delivery systems can improve the pharmacodynamic and kinetic profile of these drugs, facilitating a slow-release profile that is advantageous for improving patient outcomes [92, 131].

In acute CNS diseases such as brain tumor or stroke, coadministration of P-gp inhibitors and nanoparticles have been used to maximize the concentration of drugs at the site of injury [119]. However, the application of P-gp inhibitors in prolonged and sustained neurodegenerative diseases (i.e., Alzheimer's disease) has led to several undesired side effects,

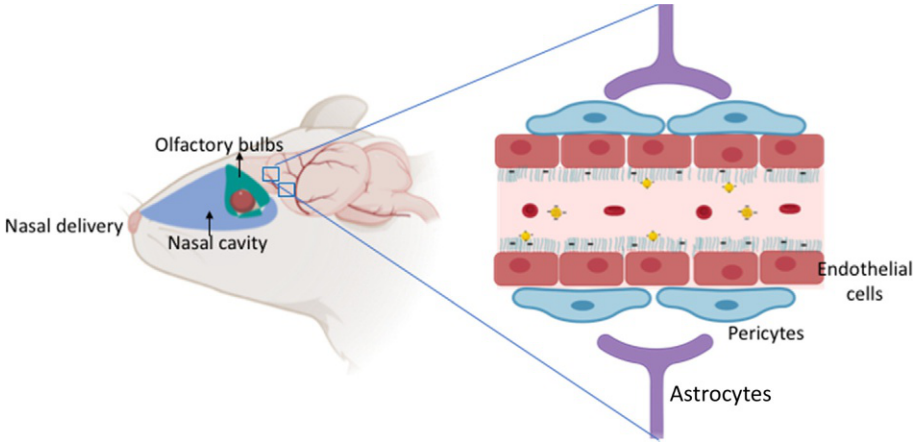

Fig. 3 Due to the complex structure and function of BBB, penetration through BBB remains the major challenge in brain drug delivery. BBB consists of at least three main cell types: endothelial cells, pericytes, and astrocytes (A). Endothelial cells of capillaries are covered by glycocalyx which is a key player of their function [134, 135], and its dysfunction leads to increased paracellular permeability. Nasal delivery is an important route of administration of nanoparticles like liposomes for targeting the brain. However, penetration through epithelial glycocalyx and the mucosal membrane is challenging for efficient drug delivery. Although penetration through BBB is difficult, several studies have used nanoparticles to deliver highly hydrophobic antipsychotic drugs such as haloperidol using dendrimers [136].

such as overdose, in patients [119, 137]. Undoubtedly, receptor-mediated endocytosis of nanoparticles into endothelial cells of the brain is another important strategy for CNS drug delivery [129]. This concept has been extensively reviewed over the last years [138].

Interestingly, recent study has indicated the involvement of heparan sulfate (HS) chains in regenerative mechanisms of various tissue types [139]. The incorporation of GAG chains into biomaterial drug delivery systems such as nano-/microparticles and hydrogels may be the next exciting development in the field of drug delivery systems. While the efficacy of such treatments remains to be elucidated, HS-containing proteoglycans have been reported to regulate BBB function, possibly by altering receptor expression [140] or stabilizing junctional complexes [141]. These findings suggest HS-/GAG-mediated signaling may be a future target for improving nanoparticle passage across biological barriers.

11. Conclusion

In the past few decades, nanotechnology is gaining an exponential interest in the field of controlled and targeted drug delivery. Given the significant progress in nanotechnology, it is now possible to synthesize nanoparticles from different materials with a wide range of physicochemical and pharmacological properties. While this has allowed nanomedicines

to overcome several of the limitations that conventional drugs encounter, they have inevitably encountered new challenges as well. From the administration site to the target tissue, nanomedicines face several obstacles that limit their efficacy and potency. To date, overcoming biological barriers remains the main challenge in the field of controlled and targeted drug delivery. As biological barriers are critical to maintaining homeostasis within the body, a better understanding of their physiology and molecular biology is critical to ensure that future drug designs do not disrupt their physiological function.

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CHAPTER 20

Regulatory pathway to introduce a nanomedicine product in the market at international level

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1. Introduction

Nanomedicine has become an important field of academic research through its clinical and commercial development in the last few decades, with direct impact on human health. Nanomedicine incorporates nanotechnology and is defined as the use of nanomaterials for diagnosis, treatment, and management of diseases [1]. Nevertheless, among different scientific and global regulatories, the concept of nanomaterial has been controversial. Some efforts were made to seek an acceptable definition of nanomedicine the fact that nanomaterials possess different physicochemical and biological properties that are attributable to their size and surface morphology, distinguishing them from drugs of low molecular weight. Such properties significantly increase a number of possibilities for developing new targeted-based drug delivery; however, some safety concerns have arisen. The design and development of nanomedicines for the drug delivery (i.e., nanoparticles containing drug), which are mostly administered by parenteral, is particularly important to the nanomedicine sector [2]. The goal of nanomedicines is to increase the therapeutic index and minimize the side effects of drugs by distributing them through different mechanisms: solubilization, passive targeting, effective targeting, and triggered release [3]. It can be regarded as a hot growth area for nanotherapeutics due to potential beneficial properties.

Nanotechnology is one of the fifth largest growth innovations to consider over the upcoming years, according to a new survey [4]. Experts at the Transparency Market Research (TMR) have followed extensive research methods to prepare a report on the 'global drug delivery systems market.' TMR analysts predict that at a 6.9% compound annual growth rate (CAGR), the market is likely to reach a value of US$ 900 billion

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through 2025 (https://www.globenewswire.com/news-release/2019/09/05/1911338/0/en/Drug-Delivery-Systems-Market-to-hit-US-900-billion-by-2025-TMR.html). The United States Food and Drug Administration (USFDA) has approved the marketing of 100 nanomedicine applications and products over the past few decades [5]. Even though the FDA has approved about 100 nanomedicines and few are in clinical trials phase, however, the current market trends in this field have not been sufficiently described.

The aim of this chapter is to provide a review of the drug delivery systems based on nanoparticles currently available in the market. It also describes potential future candidates currently being tested in clinical trials and discusses the major challenges associated with the commercial development of these systems in order to inform and refine the future development of nanomedicine. Through offering insights into the approval process and the technical and scientific criteria, we hope that this summary will help to gain an understanding of the translation path for nanopharmaceuticals.

2. Nanopharmaceutical development from laboratory to market

Pharmaceuticals focused on nanotechnology provide innovative solutions to fundamental issues in the pharmaceutical industry, ranging from low water solubility of drug to a lack of target specificity. The cost of drug discovery, design, and development will be minimized by nanotechnology with time. Nanoparticulate structures used as new and site-specific drug delivery systems, which include different carrier system like carbon nanotubes [6], nanostructured vaccines [7], polymeric micelles [8], liposomes [9], niosomes [10], nanocrystals [11], polymersomes [12], mesoporous silica [13], and dendrimeric nanostructures [14, 15].

The development and molecular understanding of innovative nanoscale materials has extended nanomedicine uses and deepened our experience and knowledge of how nanotherapeutics can be exploited in the clinic. With time, the field of nanomedicines matures and as we have progressed and in transitional phase of lab to market success. As we are in transition phase, it is very important for us to learn from our experiences in the traditional or conventional pharmaceutical industries about how one can design, manufacture, and market nanomedicines for a more effective and reliable clinical use. At present, the time frame for a product to enter the market after its initial discovery/development may take up to more than two decades [16]. Several variables present several difficulties in advertising nanomedicine, for example, because nanoscale has an unclear description of international regulatory agencies [17], nomenclature, characterization, analysis techniques [18], scaling up challenges, manufacturing costs, and toxicity [19]. To reduce the risk of failure, it is very important to thorough assess the market needs and future opportunities by experts. Some important steps along this route are briefly

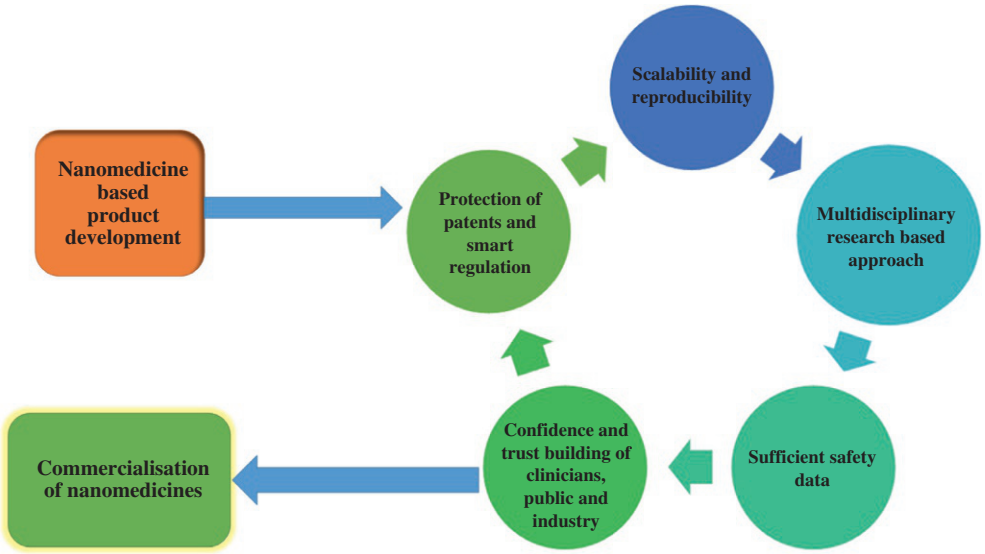

Fig. 1 Life cycle of a nanomedicine from conception to a commercialization.

discussed in the following sections, including intellectual property right, technical issues, overhead cost of the product, and also ethics and regulatory matters. Fig. 1 shows proposed journey of nanomedicines.

2.1 Intellectual property right (IPR): What can be patented

Patent law or IPR is now at the front line of drug development and nanopharmaceuticals. Patents must be filed at all levels of the drug discovery and marketing process in order to protect the intellectual properties of inventors and businesses, as well as to save money and time from being spent in conflicts and even lost legal cases. Nonetheless, it takes time to receive regulatory approval for a drug to undergo the requisite clinical trials. Eventually, with a view to the promotion of a new product, the 20-year period for patent protection (depending on the country) decreases to 12 years or less the period for commercial exclusive rights [20]. The profits earned during the exclusivity period would cover the investment made in the production of the invention. In addition, the inventor announces new technology or inventions freely for the public that would otherwise not have been revealed [21]. The technology can be freely utilized, made, sold, or imported once the patent has expired. The time left for the company to make money is, therefore, sometimes too short to invest the resources needed. On the other side, other inventors were forced to create breakthroughs that promote trade. In the recent times, the understanding and importance of patenting a concept or invention together with the technological revolution has led to numerous patents proliferating [22].

There are many potential obstacles to the intellectual property issues of nanopharmaceuticals, some of which are discussed here. The main challenge in the field of nanotechnology is to describe the word "nano" itself. Appropriate landscaping for patents in the nanotechnology arena has become a very challenging task. This becomes important when a prior art application for a patent is undertaken. Sometimes, the inventor sets broad claims for a specific patent. Under these conditions, the recruitment of well-trained nanotechnology patent examiners who would only award quality patents with the correct evaluation would need to be accomplished. These are the current regulatory issues [23].

2.2 Technical path

The transformation of research and innovation in nanomedicine into multiple opportunities for the public is determined by a number of factors, such as scale-up challenges, high cost of production, lack of capital resources, as well as market adoption, particularly with regard to trust in the security of new products. The leading patent authorities in the world have had issues since the past decade with pending patent applications related to nanomedicine. Adding to this, the prevalent uncertainty about nanotechnology identification and definition contributes to the problem of commercialization nanomedicine developments [24, 25]. Bigger company comes forward to take the responsibilities because larger firms are better equipped to market a drug from the laboratory bench. Invention is the secret to the growth of any pharmaceutical industry for sustainability as well as the development of new and superior therapies to address the limitations. Researchers carry out research, technicians are responsible for the method of manufacturing, lawyers take responsibility for the filing and protection of patents, and shareholders and existing profit streams from already marketed drugs. The status of nanomedicine as of today is only a landmark on the path to the implementation of truly innovative technology [26]. Nevertheless, future developments and implementations, planned in the coming decades, would focus on how the nature of the clinical trials could be addressed and how it would be embraced and accepted by the public.

2.3 Costs

The price over the life span of any new drug consists of time and money from the original idea and preclinical testing, industrial development and eventually regulatory approval, and the phase of commercialization and marketing. Taking into consideration the long time it takes for the product to be marketed, the relevant patents that could have expired and inflation and discount rates are equivalent to $2558 million [27]. Formulation design and optimization is an integral part of any therapeutic agent's manufacturing and marketing that is indeed a time-consuming and expensive process [28, 29]. The optimization method may involve alterations in the composition of the

formulation, the production process, the equipment, and the sizes of the sample. If such changes apply to a formulation, experiments in healthy individuals could be necessary to prove the bioequivalence of the new formulation with the older one. The implementation of these requirements does not only prevent the marketing of the new formula, but also increases the cost of the process of optimization. As a consequence, an increase will eventually result in higher product costs for the customers. Therefore, designing in vitro tests which represent information on bioavailability would be beneficial. The pharmaceutical industry has found ways to supply nanomedications to patients at affordable cost. The FDA developed a regulatory guideline to reduce the need for bioavailability studies as part of the formulation development and optimization to both immediate and modified dosage forms. The legal, ethical, and cost control methods should be considered during the development of new nanopharmaceuticals [30]. A key factor in explaining the rising costs of developing every new drug is the high rate of project failure. This is particularly relevant to the added complexity of novel new nanomedicines. It is important to remember legal, ethical, and cost control procedures during the development of new nanomedicines. The marketing of approved nanopharmaceuticals can lead to enormous profits in view of their increased efficiency. But the high failure rate has prevented many big businesses from investing in this field, and researchers and entrepreneurs still have the opportunity to seize.

2.4 Ethics

Nanopharmaceuticals ethics go hand in hand with regulatory frameworks. Ethical concerns related to governance and policies are central to respecting the dignity of people involved in nanomedicine research studies and how to protect citizens' fundamental human rights that may be uncovered to free nanoparticles in the environment. Promoting the responsible use of nanomedicine is important to protect human health and the environment. Nevertheless, there are unique ethical issues to be addressed in this scientific field, such as justice equality and independence. Nanomedicine advances in clinical trials and the assumption of general safety, in line with social and ethical appropriateness, pose some bioethical concerns, which are followings:

1. *The key factor is the risk/benefit assessment*: the determination of this ratio has its own issues, as no guidelines or specific instructions for the evaluation.
2. *Health protection*: refers to medicine as well as medical research.
3. *Informed consent*: accurate data on the diagnosis, treatment, and therapy suggested may be difficult to provide.
4. Medical and nonmedical uses: therapy and enhancement?
5. Such organizations also should be briefed by data security control board investors and independent experts.

3. The FDA's approach to the regulation of nanotechnology products (FDA rules and regulations)

The age of nanotechnology is upon us and this technological revolution is rapidly developing. Nowadays, industrial nanomedicine is in the science pipelines [31]. The FDA agrees that nanotechnology is an expanding market that has the ability to be used around across full spectrum of the FDA-approved products including medications, biological products, and medical devices. The FDA has taken several steps over the past several years to help ensure responsible development of nanotechnology products [1]. As a public health department, scientific evidence is used to control products, including cosmetics, medication, and food packaging. The FDA has long encountered the combination of promise, risk, and uncertainty that accompanies emerging technologies. In this way, nanotechnology is not different. Products can exhibit new or altered nanoscale physicochemical properties that can allow the development of new products. However, the changes in biologicals, chemicals, and other properties that can make nanotechnology applications so exciting can also be considered to determine any effects on product safety, efficacy, or other attributes. The FDA has focused on a regulatory science program for nanotechnology to help address key scientific gaps in knowledge, methods, and tools needed to control nanotechnology products [32]. In 2011, the FDA released a draft industry guideline called "Considering Whether an FDA-Regulated Product Involves the Application of Nanotechnology" to reflect the latest nanotechnology analysis and receive public comments [33]. No specific policy guideline for nanopharmaceuticals (or the classification of nanomaterials in general) had been released by the FDA until recently. Nonetheless, a report was released in August 2016 outlining general rules for all cosmetics, food ingredients, and animal feed nanomaterial products [34]. As mentioned earlier, it takes about 10–20 years to market a new pharmaceutical, but it should be noted that nanomaterial science is still a young field today [35]. Nanomedicine interactions with its biological environment (at the level of proteins, cells, tissues, etc.) rely on a complex interplay between the controllable properties of the particles and the essentially uncontrollable properties of the surrounding media [36]. A number of nano-based pharmaceuticals have now entered the market widely and are used by most patients on a daily basis. Such products come from different companies around the world and demonstrate the present and (possibly) future success of nanomaterials as therapeutic agents. The following are some of the most popular nanopharmaceuticals by type of nanoformulation discussed. These groups are: nanocrystals, liposomes and lipid-based, polymeric (including pegylated biologics, gels, and emulsions), protein-based, and metallic NPs. The FDA-approved nanomedicines are given in Table 1.

Table 1 List of FDA-approved nanomedicines.

Brand name	Disease condition	Company	Nanotechnology	Approved year
DexIron	Iron-deficient anemia	Sanofi Avertis	Iron dextran (high MW)	1957
INFeD	Iron-deficient anemia	Sanofi Avertis	Iron dextran (low MW)	1957
Adagen®	Severe combined immunodeficiency disease (SCID)	Sigma-Tau Pharmaceuticals	PEGylated adenosine deaminase enzyme	1990
Oncapar®	First-line acute lymphoblastic leukemia	Enzon Pharmaceuticals	Polymer-protein conjugates	1994
Abelcet®	fungal infections and leishmaniasis	Sigma-tau	Liposomal	1995
Doxil®	Metastatic breast cancer, ovarian cancer, multiple myeloma, and AIDS-related Kaposi's sarcoma	Janssen	Liposomal doxorubicin	1995
Copaxone®/ Glatopa	Multiple Sclerosis (MS)	Teva	Random copolymer of L-glutamate, Lalanine, L-lysine and L-tyrosine	1996
DaunoXome®	Acute myeloid leukemia, Kaposi's sarcoma	Galen	Liposomal daunorubicin	1996
DepoCyt©	Lymphomatous meningitis	Sigma-Tau	Liposomal cytarabine	1996
Feridex®/ Endorem®	Iron-deficiency anemia	AMAG pharmaceuticals	SPION coated with dextran	1996
AmBisome®	Serious fungal infections	Gilead Sciences	Liposomal amphotericin B	1997
Curosurf®/ Poractant alpha	Respiratory distress syndrome (RDS) in premature infants	Chiesi Farmaceutici	Liposome-proteins SP-B and SP-C	1999
Ferrlecit®	Iron-deficiency anemia	Sanofi Avertis	Sodium ferric gluconate	1999
Ontak®	Persistent or recurrent cutaneous T-cell lymphoma	Eisai Inc	Engineered Protein combining IL-2 and diphtheria toxin	1999
Rapamune®	To prevent the body from rejecting a newly transplanted kidney	Wyeth Pharmaceuticals	Sirolimus	2000
Renagel®	Chronic kidney disease	Sanofi	Poly(allylamine hydrochloride)	2000

Continued

Table 1 List of FDA-approved nanomedicines—cont'd

Brand name	Disease condition	Company	Nanotechnology	Approved year
Venofer®	Iron-deficiency anemia	Luitpold Pharmaceuticals	Iron sucrose	2000
Visudyne®	Macular degeneration, wet age related; myopia; ocular histoplasmosis	Bausch and Lomb	Liposomal Verteporfin	2000
Lumirem®	Used to enhance the contrast of the images obtained during MRI examinations of the stomach and Bowels	AMAG pharmaceuticals	SPION coated with silicone	2001
Megace ES®	Anti-anorexic	Par Pharmaceuticals	Megestrol acetate	2001
PegIntron®	Hepatitis C	Merck	PEGylated IFN alpha-2b protein	2001
Avinza®	Psychostimulant	Pfizer	Morphine sulfate	2002
Eligard®	Prostate Cancer	Tolmar	Leuprolide acetate and polymer	2002
Neulasta®	Neutropenia	Amgen	PEGylated GCSFprotein	2002
Pegasys®	Hepatitis B; Hepatitis C	Genentech	PEGylated IFN alpha-2a protein	2002
Ritalin LA®	Psychostimulant	Novartis	Metyhlphenidate HCl	2002
Zanaflex®	Muscle relaxant	Acorda	Tizanidine HCl	2002
Emend®	Antiemetic	Merck	Aprepitant	2003
Estrasorb™	Menopausal therapy	Novavax	Micellar Estradiol	2003
OsSatura®	Bone substitute	IsoTis Orthobiologics	Hydroxyapatite	2003
Somavert®	Acromegaly	Pfizer	PEGylated HGH receptor antagonist	2003
Vitoss®	Bone substitute	Stryker	Calcium phosphate	2003
DepoDur®	Analgesia (postoperative)	Pacira Pharmaceuticals	Liposomal morphine sulfate	2004

Macugen®	Macular degeneration, neovacular age related	Bausch & Lomb	PEGylated anti vascular endothelial growth factor aptamer	2004
Ostim®	Bone substitute	Heraseus Kulzer	Hydroxyapatite	2004
Tricor®	Hyperlipidemia	Lupin Atlantis	Fenofibrate	2004
Abraxane®	Breast cancer Non-small-cell lung carcinoma Pancreatic cancer	Celgene	Albumin-bound paclitaxel nanoparticles	2005
Focalin XR®	Psychostimulant	Novartis	Dexamethylphenidate HCl	2005
NanOss®	Bone substitute	Rti Surgical	Hydroxyapatite	2005
Mircera®	Iron-deficiency anemia	Hoffman-La Roche	Chemically synthesized ESA (erythropoiesisstimulating agent)	2007
Cimzia®	Crohn's disease: Rheumatoid arthritis, Psoriatic arthritis, ankylosing spondylitis	UCB	PEGylated antibody fragment	2008
EquivaBone®	Bone substitute	Zimmer Biomet	Hydroxyapatite	2009
Feraheme™/ ferumoxytol	Iron-deficiency anemia	AMAG pharmaceuticals	Ferumoxytol SPION with polyglucose sorbitol carboxymethylether	2009
Invega® Sustenna®	Schizophrenia	Janssen Pharms	Paliperidone Palmitate	2009
Krystexxa®	Chronic gout	Horizon	Polymer-protein conjugate	2010
Nanotherm®	Glioblastoma	MagForce	Iron oxide	2010
Marqibo®	Acute lymphoblastic leukemia	Onco TCS	Liposomal	2012
Plegridy	Multiple sclerosis	Biogen	Polymer-protein conjugate	2014
Ryanodex	Malignant hypothermia	Eagle Pharmaceuticals	Dantrolene sodium	2014
ADYNOVATE	Hemophilia	Baxalta	Polymer-protein conjugate	2015
Onivyde®	Pancreatic Cancer	Merrimack	Liposomal	2015

4. Conclusions and future perspective

Since the first approval in the 1950s, nanomedicines have been accompanied by higher efficiency and efficacy expectations compared to less complex drugs. Nanomedicine is a global trading corporation. Most nanoparticles systems are the FDA approved and are used either for the treatment or diagnosis of disease in clinics. Approval of these pharmaceutical nanomedicines has produced milestone advancements that have led to the improvement of health and human life. Industry and governments are starting to see clearly their great potential. Although, it is an emerging field with great market potentials, but the undefined clear definition is an issue. There is no any clear defined definition of nanomedicines. It is because nanotechnology represents a group of technologies with different characteristics and applications. Some innovative applications related to nanomedicine are under development or close to launch, but the process of transforming basic research into commercially viable products in nanomedicine will be long and not easy. There are many formidable challenges, including technical, legal, environmental, safety, ethical and regulatory issues, as well as combining thickets of patent claims overlapping. In order to address these challenges, the long-term price needs more public awareness of nanomedicines benefits, risks, and safety concerns.

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Index

Note: Page numbers followed by *f* indicate figures and *t* indicate tables.

C

D

E

F

G

H

I

N

O

P

Q

R

S

T

U

V

W

Y

Z

Theory and Applications of Nonparenteral Nanomedicines

Edited by
Prashant Kesharwani, Sebastien Taurin, and Khaled Greish

This book titled *Theory and Applications of Nonparenteral Nanomedicines* provides a recent overview of the nanomedicines developed to improve patient comfort and the management of various diseases and conditions.

Over the past few years, nonparenteral nanomedicines have emerged as a viable alternative and benefited from the understanding of the anatomy and physiology of delivery routes such as oral, pulmonary, nasal, ocular, and transdermal, as well as the local administration.

Beginning with a brief introduction to the nonparenteral delivery of nanomedicine and the safety and regulatory implications of the nanoformulations, further chapters discuss the physiology of the biological barriers, the specificity of the nanocarriers as well as their multiple applications.

Theory and Applications of Nonparenteral Nanomedicines helps research clinicians, researchers working in drug delivery and pharmaceutical industries, and students to understand the recent progress in the design and development of nanoformulations compatible with nonparenteral applications.

Key Features:

- Contains a comprehensive review of nonparenteral nanomedicines.
- Provides analysis of nonparenteral methods of nanomedicines including regulatory implications and future applications.
- Explores a wide range of promising approaches for nonparenteral drug delivery using the latest advancement in nanomedicine written by experts in industry and academia.

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